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Anti-Dyslipidemic Properties of Saffron: Reduction in the Associated Risks of Atherosclerosis and Insulin Resistance

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Abstract

Background: Recently herbs considered as biological and safe agents to treat, control and prevent of many health problems such as obesity and its complications.
Objectives: This study investigated protective effects of extracts from saffron stigma, petal, and their mixture on dyslipidemia, atherosclerosis, and insulin resistance in high-fat-fed obese rats.
Methods: This experimental study was performed in animal house of Birjand University of Medical Sciences, Birjand, Iran. We used systematic random sampling to divide 56 adult male rats into 8 groups with 7 rats in each group that were fed a high-fat or standard diet for 10 weeks. Then, doses of saffron stigma, petal (40 and 80 mg/kg body weight, respectively), and their mixture (80 mg/kg body weight of both) were administered orally on a daily basis for three weeks. At the end of treatment priods, we examined all biochemical parameters. Data were analyzed by valid statistical analysis.
Results: Saffron extracts markedly ($P < 0.05$) decreased the serum total cholesterol (TC, 90 ± 9.3), triglyceride (TG, 99 ± 10.5), and low-density lipoprotein cholesterol (LDL-C, 27 ± 1.1) in obese rats, while they increased the serum high-density lipoprotein cholesterol (HDL-C, 68 ± 2.2). The atherosclerosis-index (LDL/HDL, 0.39 ± 0.5), atherogenic index (TC/HDL, 1.32 ± 4.2), and the liver enzymes (ALT, AST, and ALP) were also reduced drastically ($P < 0.05$) after herbal treatments. Treatment with saffron extract significantly ($P < 0.05$) decreased the levels of leptin (0.26 ± 0.04), insulin (4.11 ± 0.1), resistin (11.1 ± 0.5) and homeostasis model assessment-estimated insulin resistance index (HOMA-IR, 20.0 ± 1.4) and enhanced the levels of circulating adiponectin (108 ± 3), potentially indicating a cardio-protective influence. Antioxidant capacity (511.63 ± 19) increased after treatment with saffron extracts, but malondialdehyde levels (1.94 ± 0.1) decreased.
Conclusions: This in vivo study demonstrated that saffron extracts, particularly the mixture of extracts from stigma and petal, ameliorated dyslipidemia in obese rats, leading to decreased atherosclerosis and insulin resistance. Our data suggested a potential therapeutic strategy against obesity and its related complications.

Keywords: Saffron, Dyslipidemia, Antioxidant, Atherosclerosis, Insulin, Adiponectin

1. Background

Obesity is one of the main health-related issues that are thought to play an important role in many human disorders including cardiovascular disease, diabetes, hypertension, osteoarthritis, and depression. The risk factors of obesity, which include genetic, environmental, behavioral, physiological, and nutritional influence, lead to a disruption of energy balance and increased fat storage (1). In nutritional obesity, diets rich in carbohydrates and/or lipids can cause individuals to become overweight and develop excessive abdominal fat. The serum levels of LDL, TC, and TG significantly increase, while HDL level shows a notable decrease in obesity. These changes in lipid profile are key risk factors for obesity and may lead to atherosclerosis or coronary heart disease (2). Furthermore, oxidative stress, induced by obesity, directly damages vascular wall cells and heart tissue (3), resulting in pathophysiology of hypertension and atherosclerosis (4).

Adipose tissue is a major source of energy for the human body and produces major adipo-cytokines such as adiponectin, leptin, and resistin polypeptides (5). Adiponectin regulates energy metabolism and plays a protective role in the development of atherosclerosis (6). Leptin reduces food intake and body weight by binding to its receptor in brain (7). Secretion of resistin hormone potentially links obesity to insulin resistance, can be up-

regulated by insulin and glucose, and its circulating levels increase in both diet-induced and genetic forms of obesity (8). Insulin hormone regulates cellular energy and macronutrient balance and plays a pivotal role in anabolic processes in fed state (9).

To date, strategies for the treatment of obesity have consisted of reduced energy diets, increased physical activity or exercise, behavioral modification, chemotherapy, surgery, and dietary supplements (10). At present, the surgical and medical therapies are not recommended because of their side effects and high costs. Natural products have been considered as organic agents with the potential for obesity treatment and even prevention (11). Saffron (Crocus sativus L.) is a perennial and stemless herb of the Iridaceae family planted widely in Iran (12). It has recently been used as a pharmacological agent for improving blood circulation, alleviating hypertension and treating coronary heart disease. Its anti-parkinson, anti-depressant, anti-cancer, anti-diabetic (13), and antioxidant properties (14) are also known. While saffron petals abound in antioxidants, a large amount of them are discarded as agricultural waste each year.

2. Objectives

The present study evaluated and compared the effects of saffron stigma, petal, and their mixture on lipid profile, liver enzymes, adipose-derived hormones, and on the risk of atherosclerosis and insulin resistance in obese rats in order to clarify the cellular mechanism behind the anti-obesity properties of saffron.

3. Methods

3.1. Preparation of Herbal Extracts

Saffron stigma and petal were dried in darkness and ground into a fine powder with a grinder (Hamilton Beach Brand, Canada). To prepare aqueous extracts, stigma and petal powders were macerated in distilled water for three days in a cool, dark place. The supernatants were subsequently filtered using Whatman No.1 filter paper and frozen in liquid nitrogen. The extraction was freeze-dried for 48 h until completely dry. To prepare the solutions for administration via oral gavages, the appropriate amount of powder was dissolved in distilled water (15). The voucher number of specimen for Saffron (Herbarium code 2669) was deposited by Dr Mohammad Ali Behdani, associate professor, Saffron research group, University of Birjand in the Herbarium of Birjand University, Birjand, Iran.

3.2. Experimental Animals

We used systematic random sampling in this experimental study (In vivo model). A total of 56 healthy male Wistar albino rats (60 days old and 200 g $\pm$ 10 g body weight) were purchased from the animal center at Birjand University of Medical Sciences (BUMS), Iran. The study was performed in Birjand, Iran from September to December 2015. All experimental procedures were approved by the animal ethical committee of BUMS, birjand, Iran (protocol n. 1392.1.1000). Animals were housed under standard conditions in temperature of 21 - 24°C and a 12: 12 hours light/dark cycle, under controlled lighting of 60 - 80 g W/cm (lights on 07.00 hour), and provided a standard laboratory diet and water ad libitum.

After two weeks, animals in high-fat diet cages were fed a high-fat diet consisting of 100 g rat food, 100 g sugar, and 200 g corn oil for 10 weeks. Then five groups of obese rats were gavaged with saffron extracts from stigmas (40 and 80 mg/kg body weight), petals (40 and 80 mg/kg body weight) or a mixture consisting of stigmas and petals (80 mg/kg) daily for three weeks. Specifically, rats were placed in distinct treatment groups: group A or control (n = 7) was fed standard diet and a received normal saline treatment; group B or the obese control (n = 14) was fed a high fat-diet and received normal saline treatment; groups C1 and C2 (n = 7) were fed a high fat-diet and given an aqueous extraction of saffron stigma (40 and 80 mg/kg, respectively); groups D1 and D2 (n = 7) were fed a high fat-diet and given an aqueous extraction of saffron petal (40 and 80 mg/kg, respectively); and group E (n = 7) was fed a high fat-diet and given a treatment consisting of combined aqueous extract of saffron stigma and petals (80 mg/kg body weight).

3.3. Body Weight

The bodyweight of each individual rat (g) was measured using a precision scale (Sartorius TE214S A, Germany) at the end of every week throughout the study.

3.4. Analysis of Blood Biochemical Parameters

At the completion of the study, blood samples were collected via cardiac puncture in sterile vials without anticoagulant for serum separation. Sera samples were analyzed for biochemical parameters such as TC, TG, LDL, HDL, urea, creatinine, alanine amino transferase (ALT/SGPT), aspartate amino transferase (AST/SGOT), alkaline phosphatase (ALP), and glucose using standard commercial kits (Technicon RA-100, USA) adapted to Selectra Auto Analyzer (Vital Scientific Spankeren, The Netherlands). The levels of adiponectin, leptin, insulin, and resistin hormones were measured using ELISA kits (Glory Science, USA) adapted to Epoch microplate reader (BioTek, Winooski, USA).

In addition, the indexes were calculated using the following formulae:

The atherogenic index = TC/HDL;

The atherosclerosis-index = LDL/HDL;

The Homeostasis Model Assessment of IR (HOMA-IR) = fasting serum insulin (μU/mL) $\times$ fasting plasma glucose (mmol/L)/22.5.).

3.5. Ferric Reducing Antioxidant Power (FRAP) Assay

To measure the total antioxidant power of plasma samples, the FRAP assay was used as described previously (16). Ferric iron was reduced to its ferrous state due to the presence of antioxidant activity and low pH levels in the samples, which led to the formation of an intensive blue ferrous-tripyridyltriazine complex. This complex could be monitored at a maximum absorption of 593 nm. In total, 2 mL of FRAP reagent consisting of 300 mM sodium acetate buffer (pH 3.6), 10 mM 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ) solution in HCl, and 20 mM $FeCl_3.6\ H_2O$ solution in a proportion of 10: 1:1 (v/v) was added to 50 μL of sample. After 15 minutes, the absorbance was measured at 593 nm.

3.6. Measurement of Thiobarbituric Acid Reactive Substances (TBARS)

Malondialdehyde (MDA) levels were measured using the thiobarbituric acid reactive substances (TBARS) method (17). Plasma samples (300 μL) were added to 3 mL TBARS reagent (7.5 g trichloroacetic acid, 187 mg TBA, and 6.25 mL chloridric acid). Next, the mixture was warmed for 20 minutes in a boiling water bath. Finally, the absorbance of the samples was determined at 532 nm.

3.7. Statistical Analysis

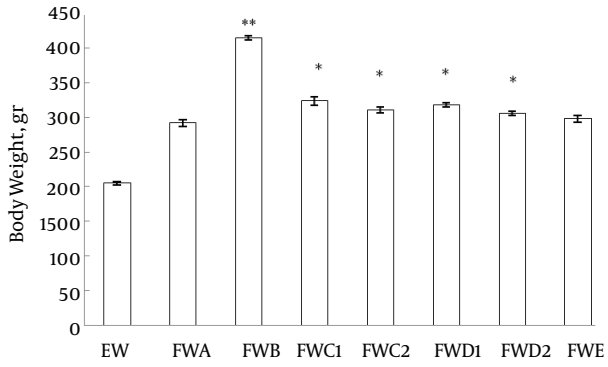
The data was analyzed using SPSS software (version 9.01). Mean values were compared using a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Normality assumption was assessed by Shapiro-Wilk test .All results are expressed as the mean $\pm$ standard error of the mean (SEM) and $P < 0.05$ was considered significant. Graphs were created using Graph Pad Prism version 5 (Graph Pad Software Inc., La Jolla, CA, USA).

4. Results

4.1. Body Weight

As shown in Figure 1, the body weight of rats in the control group (Group A) was less than rats in the obese group (Group B) at the completion of the study ($P < 0.01$). After daily treatment with saffron stigmas, petals, and mixed extracts for 3 weeks, body weight decreased in all groups (Groups C1-E, $P < 0.05$).

Figure 1. Effects of Saffron Extracts on Body Weight of Rats



Data are expressed as mean $\pm$ SEM (n = 7); values are statistically significant at **P < 0.01 (obese group vs. normal group) and *P <0.05 (saffron treated groups vs. obese group); EW, early weight (before starting the treatments); FWA, final weight of a control (standard diet and normal saline) group; FWB, final weight of a high fat-diet and normal saline group; FWC1 and FWC2, final weight of high fat-diet and aqueous extraction of saffron stigma (40 and 80 mg/Kg, respectively); FWD1 and FWD2, final weight of A high fat-diet and aqueous extraction of saffron petal (40 and 80 mg/Kg, respectively); FEW, a high fat-diet and combined aqueous extract of saffron stigma and petals (80 mg/kg body weight).

4.2. Lipid Profile

According to our data presented in Table 1, the lipid profile is different between experimental groups (Groups A-E). In group B, the serum levels of TC, TG, and LDL-C significantly ($P < 0.01$) increased while the serum levels of HDL-C decreased when compared with group A. The serum levels of TC, TG, and LDL-C in other saffron treated groups of rats (Groups C1-E) markedly ($P < 0.05$) decreased while the levels of HDL-C increased compared with the levels measured in rats from group B. Among saffron extracts, mixture of stigmas and petals affected the lipid profile of obese rats more than others and improved the lipid profile to the normal range.

4.3. Biochemical Profile

As indicated in Table 2, the serum level of glucose in rats in group B was significantly increased when compared with rats in group A ($P < 0.01$). The serum level of glucose in other saffron treated groups of rats (Groups C1-E) was significantly decreased compared with obese rats ($P < 0.05$). The mixture of extract from stigmas and petals had the most effect on serum glucose levels in obese rats. Additionally, the levels of liver enzymes (ALT, AST, and ALP) in serum samples were significantly higher in rats from the obese group than in animals from the control group ($P < 0.01$). These enzymes markedly declined to normal values in all groups after treatment with different doses of saffron extracts. The mixture of stigma and petal extract

Table 1. Effects of Saffron Extracts on Lipid Profile in Rats[a,b]

Groups	Group A	Group B[c]	Group C1	Group C2[d]	Group D1[d]	Group D2[d]	Group E[d]
TC, mg/dL	81 ± 9.9	189 ± 11	131 ± 10	122 ± 10.8	108 ± 9	95 ± 1	90 ± 9.3
TG, mg/dL	90 ± 10.5	250 ± 11	152 ± 11.6	138 ± 11.2	118 ± 1	105 ± 1	99 ± 10.5
LDL-C, mg/dL	21 ± 1.1	64 ± 1.5	35 ± 1.1	32 ± 1.8	33 ± 1.8	30 ± 1.6	27 ± 1.1
HDL-C, mg/dL	55 ± 2.1	41 ± 1.8	49 ± 2.2	56 ± 1.5	58 ± 3.2	63 ± 1.9	68 ± 2.2

[a]Group A, A standard diet and normal saline; Group B, A high fat-diet and normal saline; Groups C1 and C2, A high fat-diet and aqueous extraction of saffron stigma (40 and 80 mg/Kg, respectively); Groups D1 and D2, A high fat-diet and aqueous extraction of saffron petal (40 and 80 mg/Kg, respectively); Group E, A high fat-diet and combined aqueous extract of saffron stigma and petals (80 mg/kg body weight).
[b]Data are expressed as mean ± SEM (n = 3).
[c]Values are statistically significant at P < 0.01 (obese group vs. normal group) and P <0.05 (saffron treated groups vs. obese group) (One-way ANOVA followed by Tukey's post hoc test).
[d]Values are statistically significant at P < 0.05 (saffron treated groups vs. obese group) (One-way ANOVA followed by Tukey's post hoc test).

had appeared to be more effective at normalizing the levels of liver enzymes in rats fed a high-fat diet. Serum levels of urea and creatinine were also measured, and sera levels of both urea and creatinine were significantly increased in Group B compared with those measured in Group A (P < 0.01). After treatment with different extracts of saffron, the serum levels of urea and creatinine decreased in the treated rats rather than in the obese rats (P < 0.05).

4.4. Hormonal Profile

The serum levels of insulin and resistin hormones were significantly increased in group B as compared to group A (P < 0.01). The serum levels of these hormones in other herbal treated groups of rats (Groups C1–E) were dramatically decreased compared with rats from group B (P < 0.05). The mixture of extracts from the stigma and petal improved the increased insulin and resistin levels of obese rats better than others (Table 3). Furthermore, in Group B, the levels of adiponectin and leptin displayed a notable decrease and increase, respectively, as compared with Group A (P < 0.01). After treatments with extracts of saffron, serum levels of leptin reduced while adiponectin serum levels significantly increased (Table 3, P < 0.05). The treatment with mixture extract improved sera adiponectin and leptin of rats more in group E than in the other treated groups (C1-D2).

4.5. Index Profile

The atherogenic index as a risk factor for cardiovascular disorders (18) was significantly higher in the obese group than the control group (P < 0.01) and markedly decreased in groups treated with saffron extracts (groups C1-E) compared with the obese group (Table 4, P < 0.05). On the other hand, the atherosclerosis-index, as a risk factor for atherosclerosis (19), was significantly increased in the rats from Group B compared with the rats from Group A (P < 0.01) and rats from the groups treated with saffron extracts (C1-E) (P P < 0.05). After saffron treatments, both atherogenic and atherosclerosis indices were reduced in obese rats in Group E compared to all other groups. HOMA-IR as a factor for evaluating insulin resistance (20) increased in group B compared with group A (P < 0.01). It markedly decreased after treatment with saffron extracts in groups C1-E. The mixture of stigma and petal extracts was most effective in decreasing insulin resistance, most probably by reducing the HOMA index (Table 4, P < 0.05).

4.6. Antioxidant Properties

As observed in Table 5, anti-oxidant capacity decreased in obese rats compared to normal rats (P < 0.01). However, when rats were treated with extracts from stigma, petal, or their mixture the anti-oxidative capacity increased (groups C1-E; P < 0.05). The highest anti-oxidative effect was observed in Group E, which received a mixture of extract from stigma and petal. Our results illustrated that serum MDA increased in rats from the obese group as compared with rats from the control group and significantly decreased after herbal treatments (P < 0.05). The most effective extract was a mixture of stigma and petal as observed with previous experiments.

5. Discussion

Obesity is a common health problem in modern societies, with serious cases leading to death. Obesity can cause several disorders including hypertension, ischemic stroke, cancer, osteoarthritis, insulin resistance, and atherosclerosis. Insulin resistance is defined as a failure of target tissues (adipose, liver, skeletal, and cardiac muscle) to respond normally to insulin. Additionally, obesity leads to elevated fasting triglyceride levels, elevated postprandial triglyceride-rich remnant lipoproteins, low HDL cholesterol, and low dense LDL particles. This pattern correlates

Table 2. Effects of Saffron Extracts on Biochemical Profile in Rats[a,b]

Groups	Group A	Group B[c]	Group C1	Group C2[d]	Group D1[d]	Group D2[d]	Group E[d]
Glucose, mg/dL	90 ± 2.4	275 ± 1.9	162 ± 1.5	150 ± 1	128 ± 1.7	118 ± 2.1	110 ± 1.9
AST, u/L	192 ± 1	321 ± 10.5	310 ± 1	292 ± 1	281 ± 1	263 ± 1	216 ± 11.5
ALT u/L	79 ± 5.7	125 ± 1	109 ± 9	102 ± 6.3	100 ± 5.5	93 ± 6.9	87 ± 8
ALP, u/L	340 ± 1	622 ± 2	523 ± 1	511 ± 2	445 ± 1	402 ± 1	362 ± 2
Urea mg/d	36 ± 0.9	52 ± 0.8	48 ± 0.7	46 ± 0.7	45 ± 0.9	42 ± 0.7	40 ± 0.9
Creatinine, mg/dL	0.6 ± 0.04	1.18 ± 0.07	1.1 ± 0.03	1.01 ± 0.05	0.92 ± 0.05	0.84 ± 0.07	0.71 ± 0.04

[a]Group A, A standard diet and normal saline; Group B, A high fat-diet and normal saline; Groups C1 and C2, A high fat-diet and aqueous extraction of saffron stigma (40 and 80 mg/Kg, respectively); Groups D1 and D2, A high fat-diet and aqueous extraction of saffron petal (40 and 80 mg/Kg, respectively); Group E, A high fat-diet and combined aqueous extract of saffron stigma and petals (80 mg/kg body weight).
[b]Data are expressed as mean ± SEM (n = 3).
[c]Values are statistically significant at $P < 0.01$ (obese group vs. normal group) and $P < 0.05$ (saffron treated groups vs. obese group) (One-way ANOVA followed by Tukey's post hoc test).
[d]Values are statistically significant at $P < 0.05$ (saffron treated groups vs. obese group) (One-way ANOVA followed by Tukey's post hoc test).

Table 3. Effects of Saffron Extracts on Hormonal Profile in Rats[a,b]

Groups	Group A	Group B[c]	Group C1	Group C2[d]	Group D1[d]	Group D2[d]	Group E[d]
Insulin, μU/mL	4.13	7.11 ± 0.1	5.21 ± 0.1	5.05 ± 0.1	4.82 ± 0.1*	4.63 ± 0.1	4.11 ± 0.1
Resistin, ng/mL	7.8 ± 0.3	15.61 ± 0.7	14.5 ± 0.5	13.85 ± 0.3	12.1 ± 0.2	11.71 ± 0.4	11.1 ± 0.5
Adiponectin, ng/mL	112 ± 5.5	23 ± 6	41 ± 2.5	66 ± 4.8	89 ± 4.1*	95 ± 3.6	108 ± 3
Leptin, ng/mL	0.23 ± 0.01	0.49 ± 0.03	0.39 ± 0.05	0.37 ± 0.01	0.35 ± 0.05	0.34 ± 0.03	0.26 ± 0.04

[a]Group A, A standard diet and normal saline; Group B, A high fat-diet and normal saline; Groups C1 and C2, A high fat-diet and aqueous extraction of saffron stigma (40 and 80 mg/Kg, respectively); Groups D1 and D2, A high fat-diet and aqueous extraction of saffron petal (40 and 80 mg/Kg, respectively); Group E, A high fat-diet and combined aqueous extract of saffron stigma and petals (80 mg/kg body weight).
[b]Data are expressed as mean ± SEM (n = 3).
[c]Values are statistically significant at $P < 0.01$ (obese group vs. normal group) and $P < 0.05$ (saffron treated groups vs. obese group) (One-way ANOVA followed by Tukey's post hoc test).
[d]Values are statistically significant at $P < 0.05$ (saffron treated groups vs. obese group) (One-way ANOVA followed by Tukey's post hoc test).

Table 4. Effects of Saffron Extracts on Index Profile in Rats[a,b]

Groups	Group A	Group B[c]	Group C1	Group C2[d]	Group D1[d]	Group D2[d]	Group E[d]
TC/HDL	1.47 ± 4.7	4.60 ± 6.1	2.67 ± 4.5	2.17 ± 7.2	1.86 ± 2.8	1.50 ± 6.3	1.32 ± 4.2
LDL/HDL	0.38 ± 0.5	1.56 ± 0.8	0.71 ± 0.5	0.57 ± 1.2	0.56 ± 0.5	0.47 ± 0.8	0.39 ± 0.5
HOMA-IR	16.5 ± 1.2	86.9 ± 3.5	37.5 ± 1.5	33.6 ± 1.7	27.4 ± 1.1	24.2 ± 0.9	20.0 ± 1.4

[a]Group A, A standard diet and normal saline; Group B, A high fat-diet and normal saline; Groups C1 and C2, A high fat-diet and aqueous extraction of saffron stigma (40 and 80 mg/Kg, respectively); Groups D1 and D2, A high fat-diet and aqueous extraction of saffron petal (40 and 80 mg/Kg, respectively); Group E, A high fat-diet and combined aqueous extract of saffron stigma and petals (80 mg/kg body weight).
[b]Data are expressed as mean ± SEM (n = 3).
[c]Values are statistically significant at $P < 0.01$ (obese group vs. normal group) and $P < 0.05$ (saffron treated groups vs. obese group) (One-way ANOVA followed by Tukey's post hoc test).
[d]Values are statistically significant at $P < 0.05$ (saffron treated groups vs. obese group) (One-way ANOVA followed by Tukey's post hoc test).

strongly with cardiovascular risk as well (9). Given the important influence obesity has on the pathogenesis of many diseases scientists have attempted to provide successful treatments. Recently, there has been evidence indicating that herbs may be effective bio-agents for obesity management (21). Several medicinal plants including Platycodon Grandiflorum, Berberine, and Green Tea have anti-obesity and lipid (TG, TC and LDL) lowering properties. They also improved obesity associated metabolic problems such as cardiovascular diseases through normalizing lipid profile (22).

Saffron is a commonly used herb which its medicinal

Table 5. The Antioxidant Effects of Saffron Extracts on Obesity in Rats[a,b]

Groups	Group A	Group B[c]	Group C1	Group C2[d]	Group D1[d]	Group D2[d]	Group E[d]
FRAP	489.26 ± 15	323.48 ± 11	401 ± 9	437.62 ± 16	413.53 ± 10	477.54 ± 15	511.63 ± 19
MDA	1.71 ± 0.2	4.92 ± 0.3	3.54 ± 0.1	2.96 ± 0.2	2.71 ± 0.1	2.29 ± 0.2	1.94 ± 0.1

[a]Group A, A standard diet and normal saline; Group B, A high fat-diet and normal saline; Groups C1 and C2, A high fat-diet and aqueous extraction of saffron stigma (40 and 80 mg/Kg, respectively); Groups D1 and D2, A high fat-diet and aqueous extraction of saffron petal (40 and 80 mg/Kg, respectively); Group E, A high fat-diet and combined aqueous extract of saffron stigma and petals (80 mg/kg body weight).
[b]Data are expressed as mean ± SEM (n = 3).
[c]Values are statistically significant at $P < 0.01$ (obese group vs. normal group) and $P < 0.05$ (saffron treated groups vs. obese group) (One-way ANOVA followed by Tukey's post hoc test).
[d]Values are statistically significant at $P < 0.05$ (saffron treated groups vs. obese group) (One-way ANOVA followed by Tukey's post hoc test).

effects have been reviewed elsewhere (14, 23). In this experimental study, we examined and compared, for the first time, the effects of saffron stigmas, petals, and their mixture on body weight, biochemical and hormonal profiles, insulin resistant HOMA, atherosclerosis and atherogenic indices, and oxidative stress parameters on diet induced obesity in rats.

Figure 1 shows body weight increase in obese rats in group B (420 ± 8 g) compared with rats in group A (290 ± 6 g; $P < 0.01$). After different concentrations of saffron stigma, petal, and their mixture were administered via oral gavage, the body weight of rats in groups C1-E significantly decreased. In addition, high TC, TG, and LDL levels combined with low HDL in obese rats were markedly improved by saffron extractions in a dose-depended manner. The mixed extract had the highest potential to modulate weight and related biochemical factors in obese rats (Table 1). At these concentrations, saffron has not been shown to exhibit any toxic effects in animals, a finding supported by a study conducted by Mohajeri et al. (13). Our results indicate that treatment with saffron may be a potential course of action to reduce overweight accompanied by hyperlipidemia without detrimental side effects. Liver enzymes (ALT, AST, and ALP), urea, and creatinine improved to normal values in herbal treated rats, whereas they remained elevated in untreated obese rats. Table 2 indicates that the mixture of extracts from saffron stigmas and petals had the greatest effect on retuning sera levels of these biomarkers to normal levels (i.e., there is no statistically significant difference between group E and other saffron treated groups). In addition, our data are similar to previous reports indicating that obesity alters metabolism of proteins and increases turnover of nitrogen, resulting in high serum urea and creatinine levels (24).

Two adipo-hormones, leptin and adiponectin, are closely related to obesity and atherosclerosis. Adiponectin protects cardiovascular tissues under stress conditions through the inhibition of pro-inflammatory agents as well as through stimulation of endothelial cell responses (25). Leptin increases vascular inflammation, oxidative stress, and vascular smooth muscle hypertrophy, which may contribute to hypertension, atherosclerosis, and coronary heart disease (26). Here, we examined the effects of various saffron extracts on serum levels of these hormones in obese rats. Our results illustrated that, in overweighed rats treated with saffron (especially in group E), adiponectin levels were significantly increased while leptin levels were significantly reduced. As shown in Table 3, the extract mixture had the most impact on adiponectin-mediated cardio protection when compared with treatments of saffron stigma and petal alone. Other studies have demonstrated that some herbs lower leptin and raise adiponectin synthesis in fat tissues resulting in a measurable change in sera levels (27, 28). We further investigated the serum levels of two other hormones, insulin and resistin, which are related to obesity induced through insulin resistance (9). Resistin seems to be involved in the development of atherosclerosis in humans by promoting the formation of foam cells as well as the proliferation and migration of vascular endothelial and smooth muscle cells (9). Insulin is a key hormone that is secreted from beta-cells of pancreatic islets in response to elevated blood glucose levels and enhanced glucose transport. Several studies have shown that insulin resistance has an important role in promoting cardiovascular diseases. Our data show that serum levels of insulin and resistin were significantly increased in obese rats compared with normal rats. Treatment with saffron extracts reduced serum levels of both hormones in all treated groups (C1-E), especially rats from group E who were treated with the mixed extract of stigma and petal (Table 3).

The correlation between dyslipidemia and atherosclerosis has been widely confirmed in diagnostic practices (29). In lipid disorders, the LDL/HDL ratio (atherosclerosis indicator (and the TC/HDL ratio (atherogenic or Castelliv index (is known to be altered as compared with controls (19). Table 4 shows that both atherosclerosis and atherogenic indices were significantly increased in group B com-

pared with group A ($P < 0.01$), suggesting that obesity is correlated with an enhanced risk of heart disease. The saffron extracts reduced these indices and can therefore be considered as potentially having properties that are protective against coronary heart disease. Based on these results, we concluded that the mixed extract from stigma and petal saffron may be a promising candidate for the treatment of atherosclerosis via dyslipidemia regulation. Our study also indicated that the HOMA index increased in rats in group B, suggesting that obesity enhances the risk of insulin resistance. The mixture of saffron extracts dramatically reduced this index, and therefore, we conclude that these extracts can be considered protective against insulin resistance.

Finally, as shown in Table 5, antioxidant capacity was significantly decreased in obese rats compared with rats in group A. Oxidative stress induced by obesity significantly resolved after administering different doses of saffron stigma, petal. Among the treatments, the extract mixture had the most antioxidant capacity. MDA the end product of lipid peroxidation is a good marker of free radical-mediated damage and oxidative stress. In obese rats, elevated MDA levels decreased following administration of different concentrations of saffron stigma, petal, and their mixture. Consistent with our other findings in this report, the extract mixture had the most potential to modulate MDA levels in obese rats. In the other study Hemmati et al. (30) reported that natural honey had protective effects on dyslipidemia by increasing adiponectin and decreasing stress oxidative and AIP in rats as well as saffron. Our data also are in agreement with a previous study that Amin and Nagy were done. High fat diet induced obesity associated with a disturbed lipid profile and defective antioxidant stability; this may have implications for the progress of obesity related problems. Treatment with herbal mixture extract (consisting of chebula, Senae, rhubarb, black cumin, aniseed, fennel and licorice) improved obesity and its associated metabolic problems. In addition, this herbal mixture has antioxidant and hypolipidaemic insulin sensitizing effects similar to saffron mixture (31).

This study for the first time revealed that a new combination of saffron stigmas and petal improved dyslipidemia and its complications such as insulin resistance and atherosclerosis in over weight rats by hormonal regulation. The strong points of this study include the investigation of effects of a saffron new mixture (80 mg/kg of stigmas and petals) on lipid profile and adipo-hormones in vivo and the use of evaluating methods that directly report dyslipidemia and its complications. The weak point of this study include keeping overweight rats during experiments. Thus, we were obligated to assay parameters over shorter time periods.

5.1. Conclusion

Our results illustrated that saffron extracts, especially the mixed extract, markedly decreased the serum levels of TC, TG, and LDL-C in obese rats, while the serum level of HDL-C increased. Thus, saffron extracts appear to have anti-obesity effects. Generally, the protective impact against dyslipidemia, insulin resistance, atherosclerosis, and oxidative stress was the most noticeable in saffron treatments comprised of the mixture of stigma (80 mg/kg) and petal (80 mg/kg), followed by extracts from petal only (80 mg/kg), and finally extracts from the stigma (80 mg/kg). Therefore, these results indicate that saffron extracts may hold the potential to be considered as a novel therapeutic approach for the treatment of obesity, diabetes, and cardiovascular disorders. Further experimental investigation is needed to determine the effective dosage of the plant extracts in clinical studies. Also, a clear understanding of the molecular and cellular mechanisms underlying this observation can possibly have therapeutic applications.

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Footnotes

Authors' Contribution: Reyhane Hoshyar designed the study and drafted the manuscript. Mahdiyeh Hosseinian and Zahra Amini carried out the experiments and collected data. Majid Rajabian Naghandar, Mahdiyeh Hosseinian, Asghar Zarban, Maryam Valavi, and Masoomeh Zare Beyki assisted in analysis of data and drafting of manuscript. Omid Mehrpour completed and revised the manuscript. All authors read and approved the final manuscript.

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