Progress in Nutrition 2018; Vol. 20, Supplement 1: 140-144 DOI: 10.23751/pn.v20i1-S.6078 © Mattioli 1885

Original article

Effect of saffron on serum leptin levels in patients with metabolic syndrome, a double-blind, randomized and placebo-controlled trial study

Marzie Zilaee[1, 2], Mohammad Soukhtanloo[3], Majid Ghayour-Mobarhan[4], Maryam Shemshian[4], Maryam Salehi[5], Gordon A.A. Ferns[6]

[1]Nutrition & Metabolic Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; [2]Nutrition Department, Faculty of Paramedicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; [3] Biochemistry Department, school of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [4]Cardiovascular Research Center, school of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran - E-mail: Ghayourm@mums.ac.ir; [5]Department of Community Medicine, school of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [6]Institute for Science & Technology in Medicine, Thornburrow Drive, University of Keele, Stoke on Trent, Staffordshire, ST4 7QB, UK

Summary. *Background:* Metabolic syndrome is a risk factor for cardiovascular disease and diabetes mellitus. A decreased level of serum leptin is reported for obese populations. Beneficial effects of saffron on human health, including appetite-regulation, have been reported previously. The aim of this study was to investigating the effect of saffron supplementation on serum leptin levels in patients with metabolic syndrome. *Material and method:* Patients with metabolic syndrome were randomly divided into two groups; a case group, receiving saffron and a control group, receiving placebo. Concentration of serum leptin was measured at baseline and after 12 weeks of the start of study. SPSS software was used to analyze the data. *Results:* There was a borderline (p=0.05) significant difference in serum leptin before and after treatment with saffron, but not in the placebo group. There was a significant difference in serum leptin concentrations between the groups (p=0.001). *Conclusion:* Saffron supplementation has no significant effect on serum leptin levels in patients with metabolic syndrome.

Key words: leptin, saffron, metabolic syndrome

List of abbreviations

Metabolic Syndrome (MS)
Cardiovascular Disease (CVD)
American Heart Association (AHA)
High Lipid Diet (HLD)
High Density Lipoprotein (HDL)
Low Density Lipoprotein (LDL)
Very Low Density Lipoprotein (VLDL)

Introduction

The multiple sclerosis (MS) is a clustering of cardiovascular risk factors that include the glucose intolerance, obesity, dyslipidaemia and hypertension. Several definitions for MS have been used according to criteria recommended by different organizations (1, 2) and studies show that people with MS are at twice the risk of developing atherosclerotic cardiovascular disease (CVD) compared with those without the syndrome (3, 4). MS has a high prevalence in developed countries. The high death rate associated with CVD underscores the essential need for effective methods to treatment the many disorders classified as CVD (1, 4). Thus, diagnosis and treatment of the MS is important for prevention and even treatment of the CVD.

Many regulatory hormones contribute to the regulation of metabolic processes. One of these hormones is leptin, which is an adipocyte-specific secreted pro-

tein that has profound effects on appetite and energy expenditure. Some studies have been shown that a deficiency of this protein is related to the development of obesity, and perhaps metabolic syndrome (5). Studies have shown that some medicinal plants can reduce the complications associated with the metabolic syndrome, including dyslipidemia and hypertension. One of these medicinal plants is saffron (*Crocus sativus*), which is a bulbous perennial of the iris family (*Iridaceae*) valued for its golden-colored, pungent stigmas, which are dried and used to flavor and color foods (6). The active constituents of saffron include many secondary metabolites, such as crocin and its derivatives, picrocrocin and safranal (7). Saffron contains more than 150 volatile and aroma-yielding compounds (7, 8). it had been reported to have antioxidative, anti-inflammatory, anticancer and hypolipidemic effects (9).

In some studies the appetite-reducing effect of saffron is reported that may be related to some hormones that involves in appetite regulation, such as leptin (5).

For investigating the effects of saffron on serum levels of leptin and appetite regulation in patients with metabolic syndrome, we designed a double-blind, randomized and placebo-controlled trial study.

Material and Methods

Study design

A 12-week randomized, double-blind and placebo-controlled clinical trial was used to test the hypothesis. The investigation was conducted in the Nutrition Clinic of Qhaem Hospital, Mashhad, Iran.

A total of 76 subjects with metabolic syndrome (defined by the International Diabetic Federation Criteria 2005), aged 18-75 years who visited the nutrition clinic of Qhaem Hospital were recruited. Participants were provided with information about the study both verbally and using information sheets. Those not in the mentioned age range, with other known systemic diseases and also pregnant and lactating women were excluded from the study. All patients provided written informed consent and the protocol satisfied Mashhad University of Medical Sciences Ethics Committee requirements.

Patients were randomly allocated to 2 groups with 38 cases in each group by using a computer-generated code as: Case group, receiving a capsule of saffron 100 mg/day (BD) and control group, receiving a capsule of placebo (BD). All patients for a 12-week period were given dietary advice based on the American heart association (AHA) guidelines. Compliance was assessed every three-weeks by counting capsules; those subjects who did not take their capsules regularly or were intolerant to the medication were excluded from the study. Anthropometric factors (including weight, height and waist circumference) and lipid profile were determined in all patients at baseline by routine methods. Blood samples were collected in the morning after a 12-hour fasting from each subject. Hemolyzed samples were excluded from analysis. After separation, aliquots of serum were frozen at –80°C until analysis for serum parameters and leptin. A questionnaire was used to collect information on socio-demographic status, occupation, smoking behavior, medical history and medication. Physical activity level was self-reported as mild, moderate and heavy.

Saffron capsule preparation

Crocus sativus L. stigma was obtained from Novin Saffron Co. (Mashhad, Iran), and was formulated as a capsule containing 50 mg of dried saffron stigma. Placebo capsules were matched for size, shape and volume of content and manufactured by the same company.

Statistical analysis

All data were presented as mean ±S.D in each group. Data were assessed for normality by using the Klomogrov-Smirnov test. Paired sample t tests and independent sample t tests were used for data analysis. A P-value< 0.05 was considered as statistically significant. All statistical analyses were performed using SPSS statistical software package (version 16.0; SPSS Inc., Chicago, IL). To compare the demographic data Fisher'sexact test or chi-square test was performed.

Results

Seventy subjects (35 individuals from the case group and 35 controls) completed the trial and three

participants in each group were excluded; 3 due to the possible symptoms of saffron induced allergy, 1 due to pregnancy and others due to not taking their capsules regularly. No significant differences were identified between patients randomly assigned to the case or control group regarding the baseline characteristics (p>0.05) (Table 1).

Results of the comparison of the serum leptin of the case and control groups are shown in figure 1. The mean concentrations of serum leptin level at baseline (week 0) and the end of study (week 12) were significantly higher in the case group (P-value=0.05) but there was no significant change in the control group (P-value=0.68). Independent sample T test showed that concentration of leptin at the end of study was significantly higher in case compared to the control group (43.83±5.17 vs 34.59±8.71, P-value=0.001).

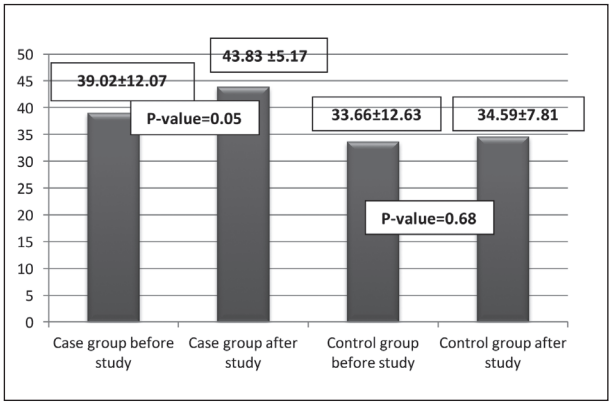

Figure 1. Comparison of serum leptin concentrations between groups before and after study intervention.
Values are expressed as mean±SD. Paired sample T test and independent T test was used for comparing the serum leptin level in case and control groups. Data showed that there is statistical significant difference between groups (P-value<0.0001) in regard to serum leptin level, before and after study.

Table 1. Baseline characteristics

	Case group	Control group
Women (n)	30	31
Men (n)	5	4
Age (year)	42.19±11.52	43.60±9.05
Weight (kg)	80.63±11.65	79.30±16.43
Height (cm)	161.65±9.53	161.90±7.30
WC (cm)	105.76±9.01	103.36±12.09
HC (cm)	115.61±9.49	112.60±10.89
Smokers (%)	11.5	10
Diabetics (%)	23.1	20
Hypertensive (%)	23.1	23.3
Hyperlipidemic (%)	23.1	23.3
Triglycerides (mg/dl)	139.76±70.14	139.00±73.52
Total cholesterol (mg/dl)	199.15±27.30	177.16±33.34
HDL-C (mg/dl)	39.03±5.34	39.13±8.00
LDL-C (mg/dl)	120.03±30.01	125.16±22.33

WC, waist circumferences; HC, hip circumferences; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBS, fasting blood sugar; DBP; diastolic blood pressure, SBP; systolic blood pressure. Values are expressed as mean±SD, or median and interquartile range. Chi-square and independent sample T test were used to compare qualitative and quantitative (normal and non-normal) variables, respectively

Comparisons of the lipid profiles and anthropometric measurements between case and control groups are shown in table 2. Results showed that in both the case and control groups, there was a significant reduction in weight (P=0.001), waist circumference (P-value=0.001) and hip circumference (0.001), and there was no significant difference between two groups. In regard to lipid profile, triglyceride level was decreased significantly in both groups (P-value=0.001 and 0.006 in case and control groups respectively), but there was no significant difference between two groups. Saffron supplements was associated with a significant reduction in total cholesterol (P-value=0.001), but no significant effect in the control group (P-value=0.189).

The serum level of high density lipoprotein (HDL) was increased significantly during the study period (P-value=0.013 and 0.009 in case and control groups respectively), but the increase in the two groups did not differ significantly. The concentration of low density lipoprotein (LDL)-cholesterol in the saffron and control group were significantly decreased (P-value=0.005 and 0.021 in case and control groups respectively), but this reduction was significantly greater in the case group; thus saffron lead to a more reduction in LDL level.

Table 2. Comparison the lipid profile and anthropometric measurements between case and control groups

	Case group		Control group		Independent sample T test (P-value) (case v control groups)
	Week 0	Week 12	Week 0	Week 12	
Weight (kg)	80.63±11.65	78.54±10.97	79.30±16.43	77.56±17.35	0.80
WC (cm)	105.76±9.01	103.00±9.02	103.36±12.09	101.03±12.69	0.51
HC (cm)	115.61±9.49	112.96±9.08	112.60±10.89	110.56±11.19	0.38
Triglycerides (mg/dl)	139.76±70.14	96.88±37.73	139.00±73.52	107.60±43.98	0.33
Total cholesterol (mg/dl)	199.15±27.30	169.39±35.87	177.16±33.34	167.36±37.03	0.83
HDL-C (mg/dl)	39.03±5.34	43.00±9.97	39.13±8.00	43.46±6.50	0.72
LDL-C (mg/dl)	120.03±30.01	97.65±25.88	125.16±22.33	113.00±26.56	0.03

LDL: low density lipoprotein- TG: triglyceride- FBS, HDL: high density lipoprotein- WC: waist circumference- HC: hip circumference
Values are expressed as mean±SD. Independent T test was used for comparing the lipid profile and anthropometric measurements in case and control groups. Data showed that there is not statistical significant difference between groups (P-value>0.05) in regard to lipid profile and anthropometric measurements, except regard to LDL-C.

Discussion

In present study we investigated the effect of saffron supplementation on serum leptin level in patients with metabolic syndrome and compared with control subjects. Our results indicated that saffron had a borderline significant effect on the serum leptin level after 12 weeks supplementation and the effect on serum leptin was significant different between the case and control groups.

Lipid profile and anthropometric measurements between two groups, except LDL-C did not differ significantly. The improvement in lipid profile and also decrease in anthropometric parameters (weight, waist and hip circumference) in two groups may be a result of diet and advice based on AHA in our study.

This study may be improve with a greater sample size and in three groups plus a negative control that the only intervention in this group can be diet, for control the effect of diet on serum leptin level and other parameters. Linear regression showed that there was no significant correlation between leptin with lipid profile and anthropometric measurements. To our knowledge, this is the first study of the effect of saffron on anthropometric measurements and lipid profile in human and the first study of the effect of saffron on serum leptin level.

The effects of saffron on body weight and lipid profile have been studied in animal models.

Zheng and colleagues (2005) administered crocetin to rabbits and determined their plasma lipid levels. Rabbits were randomly assigned to three different diets (a standard diet, a high lipid diet (HLD), or a high lipid+crocetin diet) for eight weeks. The HLD group developed hypercholesterolemia and atherosclerosis, while the crocetin-supplemented group decreased the negative health effects of a high lipid diet. Results did not show any significant difference in plasma lipid between the HLD and crocetin groups (10). Sheng and colleagues in 2006, reported that in diet-induced hyperlipidemic rats, treatment with crocin significantly reduced serum triglyceride, total cholesterol, LDL cholesterol and very low density lipoprotein (VLDL)- cholesterol level in the daily dose range of 25 to 100 mg/kg (11).

In the 2005 study, He and colleagues administered crocetin and crocin. The control group and hyperlipidemic diet group were compared with the crocetin groups, fed 25, 50, and 100 mg/kg/day. The results showed inhibition of increased total serum cholesterol, LDL, and very low density lipoproteins (VLDL) that were significant compared to the hyperlipidemic diet fed group; but crocin did not show any significant effects on serum HDL-C and body weight in quails (12).

In the 2007 study by the same group, administration of crocetin in fructose-fed rats significantly decreased serum triglycerides and LDL-C in the high-dose crocetin group. No significant difference in body weight was observed among groups at the end of the experiment (13).

In the current study, saffron decreased serum TC and LDL-C in patients with metabolic syndrome. Hence, this effect may be related to the inhibitory effect of crocin on pancreatic lipase activity.

We suggested that it is better to determine the effect of saffron on the expression of the gen of leptin in future works (instead of serum level as a limitation in our study). But in a logical sense, it is better to do this pilot study first on serum levels and after, determine the effect of saffron on the expression of the gen of leptin.

In summary, our results showed that saffron appeared to be associated with an increased the level of leptin, which may be the cause of decreasing the anthropometric measurements, but reduction of these parameters had no significant in both groups. But saffron in present study had no more effective impact on anthropometric measurements and lipid profile, except LDL-C, rather than control group. Thus present study indicate that saffron has a mildly effects on some parameters of metabolic syndrome and further studies need to be done for a more comprehensive measurements of the all metabolic syndrome parameters and as a multicenter manner.

Acknowledgement

This study has been financially supported by a grant from Mashhad University of Medical Sciences (MUMS). We would like to thank all the patients for participating in this study.

References

1. Grundy SM. Metabolic syndrome pandemic. Arteriosclerosis, thrombosis, and vascular biology 2008; 28(4): 629-36.
2. Cameron AJ, Shaw JE, Zimmet PZ. The metabolic syndrome: prevalence in worldwide populations. Endocrinology and metabolism clinics of North America. 2004; 33(2): 351.
3. Nesto RW. The relation of insulin resistance syndromes to risk of cardiovascular disease. Reviews in cardiovascular medicine 2003; 4: S11.
4. Gale EA. Should we dump the metabolic syndrome?: Yes. Bmj 2008; 336(7645): 640-.
5. Matkovic V, Ilich J, Badenhop N, Skugor M, Clairmont A, Klisovic D, et al. Gain in body fat is inversely related to the nocturnal rise in serum leptin level in young females. Journal of Clinical Endocrinology & Metabolism 1997; 82(5): 1368-72.
6. Abdullaev FI. Cancer chemopreventive and tumoricidal properties of saffron (Crocus sativus L.). Experimental Biology and Medicine 2002; 227(1): 20-5.
7. Rios J, Recio M, Giner R, Manez S. An update review of saffron and its active constituents. Phytotherapy Research 1996; 10(3): 189-93.
8. Abe K, Saito H. Effects of saffron extract and its constituent crocin on learning behaviour and long-term potentiation. Phytotherapy Research 2000; 14(3): 149-52.
9. Shen XC, Qian ZY. Effects of crocetin on antioxidant enzymatic activities in cardiac hypertrophy induced by norepinephrine in rats. Die Pharmazie-An International Journal of Pharmaceutical Sciences 2006; 61(4): 348-52.
10. Zheng S, Qian Z, Tang F, Sheng L. Suppression of vascular cell adhesion molecule-1 expression by crocetin contributes to attenuation of atherosclerosis in hypercholesterolemic rabbits. Biochemical pharmacology 2005; 70(8): 1192-9.
11. Sheng L, Qian Z, Zheng S, Xi L. Mechanism of hypolipidemic effect of crocin in rats: crocin inhibits pancreatic lipase. European journal of pharmacology 2006; 543(1): 116-22.
12. He S-Y, Qian Z-Y, Tang F-T, Wen N, Xu G-L, Sheng L. Effect of crocin on experimental atherosclerosis in quails and its mechanisms. Life sciences 2005; 77(8): 907-21.
13. Xi L, Qian Z, Xu G, Zheng S, Sun S, Wen N, et al. Beneficial impact of crocetin, a carotenoid from saffron, on insulin sensitivity in fructose-fed rats. The Journal of nutritional biochemistry 2007; 18(1): 64-72.

Correspondence:
Majid Ghayour-Mobarhan
Cardiovascular Research Center,
Faculty of Medicine, MUMS, Mashhad, Iran
Tel. +98-511+8828573
Fax +98-511-8828574
E-mail: Ghayourm@mums.ac.ir