Clinical Nutrition ESPEN 34 (2019) 130–136

Contents lists available at ScienceDirect

Clinical Nutrition ESPEN

journal homepage: http://www.clinicalnutritionespen.com

Original article

Effect of saffron (*Crocus sativus L.*) on lipid profile, glycemic indices and antioxidant status among overweight/obese prediabetic individuals: A double-blinded, randomized controlled trial

Elham Karimi-Nazari [a], Azadeh Nadjarzadeh [a, b], Roghayyeh Masoumi [a], Ameneh Marzban [c], Seyed Ahmad Mohajeri [d], Nahid Ramezani-Jolfaie [a, b, *], Amin Salehi-Abargouei [a, b]

[a] Nutrition and Food Security Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[b] Department of Nutrition, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[c] Student Research Committee, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[d] Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

ARTICLE INFO

Article history:
Received 30 March 2019
Accepted 19 July 2019

Keywords:
Saffron
Crocus sativus L.
Glycemic markers
Lipid profile
Oxidative stress
Prediabetes

SUMMARY

Objective: To investigate the effects of saffron (*Crocus sativus* L.) on lipid profile, glycemic and antioxidant status in overweight/obese individuals with prediabetes.
Methods: In this randomized, double-blind, placebo-controlled trial, the prediabetic patients were randomly assigned to receive saffron (15 mg/d) pills or placebo for eight weeks. Serum levels of lipid profile, fasting blood sugar (FBS), glycosylated hemoglobin (HbA1c), blood urea nitrogen (BUN), creatinine, and diphenylpycrylhydrazyl (DPPH) radical scavenging activity were assessed biochemically at baseline and at 8 weeks after treatment. The adverse events, if any, were also recorded.
Results: Seventy-five of participants (36 in treatment and 39 in placebo groups) completed the study. Within-group comparisons revealed a significant effect of saffron supplementation on FBS (118.11 ± 3.55 vs. 109.14 ± 6.23), HbA1c (5.85 ± 0.12 vs. 5.70 ± 0.11), and DPPH (11.06 ± 3.24 vs. 13.46 ± 3.33) levels ($P < 0.005$ for all). In adjusting models, there was a significant reduction in FBS by −7.97 mg/dL, and HbA1c by −0.15% in saffron group compared to placebo. Moreover, saffron intake tended to increase in DPPH radical scavenging activity (2.4% vs. −0.85% in saffron and placebo groups, respectively). However, no significant changes in anthropometric measures, lipid profile, and renal markers were observed after saffron intake compared with placebo.
Conclusion: Saffron supplementation could improve glycemic and antioxidant indices in overweight/obese individuals with prediabetes, however, no beneficial effect was observed on lipid profile and anthropometric parameters. (IRCT20120913010826N19).

1. Introduction

Diabetes mellitus is a chronic metabolic disorder that affect nearly every organ in the body [1]. The prevalence of diabetes and its complications is increasing worldwide, and is the seventh leading cause of disability in the world that contributes to the deaths of two million adults per year [2–5]. Diabetes complications include disturbance in lipid profile, hyperglycemia, hypertension, obesity, and increased oxidative stress which are associated with vascular complications [6–8].

Before diabetes is diagnosed, there is a period called 'prediabetes' in which blood sugar levels are high, but not high enough to be diagnosed as diabetes. It is defined as having impaired fasting blood glucose (100–125 mg/dL), impaired oral glucose tolerance test (OGTT) (140–199 mg/dL), and/or hemoglobin A1c (HbA1c) value of 5.7–6.4% [9]. Prediabetes is not only a potential risk factor for developing type 2 diabetes, but also can lead to cardiovascular disease [10].

* Corresponding author. Department of Nutrition, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
E-mail address: Ramezani.n.j@gmail.com (N. Ramezani-Jolfaie).

https://doi.org/10.1016/j.clnesp.2019.07.012
2405-4577/

Data from the Nurses' Health Study suggest that 90% of type 2 diabetes in women can be attributed to five risk factors including excess weight, lack of exercise, consuming unhealthy diets, smoking, and drinking alcohol [11]. It is thus important to manage patients with prediabetes through lifestyle and behavioral modifications such as increased physical activity and consuming healthy dietary components to prevent or delay the progression of prediabetes to diabetes [12].

There are some herbs and spices which have been claimed to have blood sugar lowering properties, and so can be useful for people with or at high risk of type 2 diabetes. A number of clinical studies in recent years showed that there are potential links between herbal therapies and improved blood glucose control, lipid profile, blood pressure, endothelial function, cardiovascular risk factors, and renal function [13–20]. More than 400 plants and compounds have been evaluated for their antidiabetic activity in in vivo and in vitro studies [21].

Saffron (*Crocus sativus* L.), a common spice in Persian cuisine, is reputed to be the most expensive traditional spice for years and widely used not only for its taste and aroma, but also for its health benefits [22]. Saffron which is native to Iran, contains compounds (e.g. crocins, safranal, crocetin, and picrocrocin) known to provide health benefits [23]. It also contains several secondary metabolites such as terpenes, flavonoids, anthocyanins, and carotenoids [24]. Previous studies have demonstrated various pharmacological effects of saffron and its active constituents including anti-oxidant [25–27], anti-tumor [28], memory and learning enhancing [29], neuroprotective [30], analgesic and anti-inflammatory [31], anticonvulsant [32], antianxiety [33], antidepressant [34], antihypertensive [35], hypolipidemic [36], and insulin resistance-reducing effect [37]. Moreover, saffron has been found to reduce the levels of liver enzymes in male rats that suffered from fatty liver disease [38].

Although some human studies have examined the efficacy of saffron in patients with type 2 diabetes [18–20,22], to our knowledge, the clinical trials in overweight/obese subjects with prediabetes are lacking. We hypothesized that saffron supplementation would help improve anthropometric measures, glycemic control, blood lipids, and antioxidant status in overweight/obese individuals with prediabetes. Therefore, the present randomized controlled clinical trial (RCT) was designed to investigate the proposed benefits of saffron on cardiovascular markers among overweight or obese individuals with prediabetes.

2. Materials and methods

2.1. Study design and population

This randomized, double-blind, placebo-controlled clinical trial was conducted with participation of prediabetic patients in Imam Ali Clinic, Yazd, Iran during March to December 2017. The diagnosis of prediabetes was based on medical records, using the American Diabetic Association (ADA) criteria: fasting blood glucose (FBG) 100–125 mg/dL or oral glucose tolerance test (OGGT) 140–199 mg/dL or HbA1c 5.7–6.4% [9]. Participants aged 40–60 years with body mass index (BMI) of 25 kg/m^2 or above were recruited. Patients were excluded if: 1) they were pregnant or lactating; 2) lack of interest in cooperation; 3) change in diet or physical activity; 4) having other diseases before admission to study or catching them during the follow up period; 5) consuming less than 30% of pills. Eighty eligible patients were randomly (1:1) allocated into saffron or placebo groups using a computerized random number table and were followed up for 8 weeks. Both investigators and participants were unaware of intervention allocation and remained blind to the treatment until all analyses were completed.

The ethics committee of Yazd University of Medical Sciences approved the study, and informed written consent was obtained from all participants. The trial was registered at the Iranian website (www.irct.ir) for registration of clinical trials as IRCT20120913010826N19.

2.2. Intervention and compliance

The total saffron extract, containing crocins and other ingredients such as safranal and picrocrocin was prepared using ethanol-water solvent according to the method described in our previous study [39]. Dried saffron extract was applied for preparation of saffron pills with total weight of 250 mg (15 mg of saffron extract and 235 mg of lactose, magnesiumstearat, and sodium starch glycolate) which manufactured by the Pharmacy School, Mashhad, Iran. The saffron pills in our study contained all active constituents of saffron stigmas including crocins, safranal and picrocrocin. Crocins are the most important active ingredients in saffron extract. Thus, the extract was standardized according to the crocins content. The analysis of crocins and standardization of the pills were performed using HPLC-UV method at 440 nm (λ max of crocins) on a Younglin (South Korea) Acme 9000 system consisting of a SP930D solvent delivery module, a SEMV50A solvent mixing vacuum degasser, a Column Oven CTS30, a UV730 dual wavelength UV/VIS detector, and an ODSA C18 (4.6 $\times$ 150 mm, 5 μm) column. The data analysis was performed using the Autochro-3000 software. A gradient method was used to chromatographically determine the crocins. The analytical method was reported in our previous study [40], and according to the HPLC-UV analysis of the saffron pill, 6 types of crocins were detected. The total amounts of crocins were discerned from the sum of the area under the curve of all the crocins in the chromatogram.

The placebo pills were completely similar to the saffron pills in shape, size and color and also were placed in the similar pocket which had contained the saffron pills to have a smell of saffron. During 8 weeks of the study period, the saffron and placebo pills were given to both groups monthly and were required to consume 1 pill per day. The last packages of pills were checked at the end of the month and the number of remaining pills was counted. Thereafter, new packages were delivered to patients by simultaneously providing usual diabetes care such as patient motivation, lifestyle management and healthy eating consultation. Participants were asked to keep their usual lifestyle including medical nutrition therapy and physical activity level during the study period. To assess patients' compliance during the entire study period, they were checked weekly using telephone calls and pill count. Also, patients were monitored weekly for recording any occurrence of adverse events.

2.3. Study outcomes

The primary outcomes of this study were as follows: levels of glycemic markers [fasting blood sugar (FBS), and glycosylated hemoglobin (HbA1c)]; lipid profile [total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and triglyceride (TG)]; antioxidant marker [diphenylpycrylhydrazyl (DPPH)]; blood urea nitrogen (BUN), and creatinine (Cr); as well as anthropometric indices [weight, BMI and waist circumference (WC)], as our secondary outcomes.

2.4. Data collection and measurements

Measurements of study variables were made at baseline and at 8 weeks following treatments. All testing were performed in the morning, after the subjects had been fasting for at least 12 h. Height

and weight were measured using a standard stadiometer and electronic scale, respectively. BMI was calculated as weight in kilograms divided by height in meters squared. WC was obtained at end-expiration and measured midway between the base of the rib cage and the superior iliac crest.

Dietary intakes of energy and macronutrients were determined using a 3-day food diary at the beginning and at the end of the study, and data were analyzed using Nutritionist IV software (First Databank, San Bruno, CA, USA), modified for Iranian foods. The levels of physical activity of participants was assessed by an Iranian version of International Physical Activity Questionnaire (IPAQ) and were expressed as metabolic equivalents minute per week [41]. This assessment was repeated at the end of the study to discover whether participants had any noticeable changes in physical activity levels.

2.5. Biochemical analysis

Blood samples were collected from all patients at the beginning and at the end of the study after 12 hr overnight fasting. The samples were immediately centrifuged (Hettich D-78532; Hettich GmbH) at 3500 rpm for 10 min to separate serum, and stored at −80 °C until analysis. Commercial kits were used to measure the levels of lipid profile, FBS, BUN, and Cr (Pars Azmoun.co kit, Tehran, Iran). The measurements were performed by autoanalyzer (Hitachi 911, Japan), using enzymatic and colorimetric method. Serum concentration of HbA1c was also measured using Elisa kit (Bioassay Technology Laboratory, Elisa kit). The DPPH reduction assay was used to measure serum DPPH radical-scavenging activity as follows [42]; 0.1 mL of deproteinized serum (with adding 100 μl acetonitrile solution to serum and centrifuging for 5min) in acetate buffered solution (10 μM, PH = 7.8) was incubated in the methanolic solution of DPPH (0.1 mM). After 30 min in room temperature, the absorbance at 517 nm was measured in triplicate (Epoch, England). Eventually, the free radical DPPH-scavenging activity was calculated by following formula: Activity [% of DPPH reduction] = [(A−Ax)/A] × 100%, where A and Ax stand for the absorbance of DPPH solution with methanol, and the absorbance of a DPPH solution with serum, respectively.

2.6. Statistical analysis

We used the Kolmogrov-Smirnov test to examine the normal distribution of variables. Continuous outcome variables were compared between the saffron and placebo groups using independent samples t-tests and differences in qualitative variables were analyzed using chi-square test. Within-group treatment effects were also analyzed based on the paired samples t-tests. To evaluate if the magnitude of the change depended on the baseline values, we considered all analyses on baseline values to avoid the potential bias. These adjustments were conducted using analysis of covariance (ANCOVA). All Analyses were performed in an intention-to-treat manner. Data are presented as mean ± standard deviation (SD). All statistical analyses were conducted by using the SPSS, version 17 (SPSS Inc. Chicago, Illinois, USA). An alpha value of 0.05 was used to determine statistical significance.

3. Results

3.1. Baseline characteristics of participants and intervention compliance

Seventy-five persons (36 in treatment and 39 in placebo groups) completed the entire study protocol as shown in Fig. 1. Subjects enrolled in the study were prediabetic patients with mean age of 57.9 years who were overweight or obese (BMI ≥ 25). Mean compliance with treatment was 90% for the intervention and 86% for placebo groups based on the remained pills at the end of the study. No adverse reactions were reported by the subjects in either group. Baseline characteristics of participants are summarized in Table 1. No significant difference was observed in demographic data between the groups, as well as physical activity levels at baseline and at the end of the study. Over the intervention period, there was also no significant change in anthropometric measures between treatment and placebo ($P > 0.05$).

3.2. Dietary assessment

Dietary intake derived from a 3-day food diary at baseline and at the end of the study is shown in Table 2. Dietary intakes of energy and macronutrients of patients did not change from baseline to the end of the study in both saffron and placebo groups ($P \geq 0.08$); indicating dietary habits of the subjects were maintained throughout the entire study period. There were also no significant differences in dietary components between study groups.

3.3. Blood lipids, glucose and HbA1c

Means of FBS, HbA1c and lipid profile in treatment and placebo groups are reported in Table 3. When we compared the effect of saffron and placebo, we failed to find any significant effect of this treatment on blood lipids between the intervention and control groups ($P > 0.05$). However, TC concentrations tended to be lower after consuming saffron (−2.13 ± 7.33 mg/dL; $P = 0.09$). Within-group comparisons revealed a significant reducing effect of saffron supplementation on FBS levels (118.11 ± 3.55 vs. 109.14 ± 6.23 mg/dL; $P < 0.005$), and HbA1c (5.85 ± 0.12 vs. 5.70 ± 0.11; $P < 0.005$). In adjusting models, there was also a significant reduction in FBS by −7.97 mg/dL, and HbA1c by −0.15% in saffron group compared to placebo ($P < 0.005$ for all).

3.4. BUN, Cr and DPPH

Results regarding the effect of saffron on serum levels of renal and antioxidant markers are given in Table 4. In the saffron group, we observed a mean increment of 2.40% in DPPH after 8 weeks of study ($P < 0.005$), whereas in the placebo group, we observed a mean reduction of 0.85% in DPPH ($P = 0.01$). A significant trend toward increasing the DPPH radical scavenging activity was also observed after saffron supplementation compared with placebo intake ($P < 0.005$). The means of BUN and Cr exhibited no significant differences by 8 weeks of saffron supplementation compared with placebo.

4. Discussion

In the present study, the effects of 8 weeks of saffron supplementation on blood lipids, glycemic and antioxidant status and also anthropometric measures were assessed in overweight/obese individuals with prediabetes. Our findings showed that compared with placebo, saffron (15 mg daily) significantly decreased serum levels of FBS and HbA1c, and increased DPPH radical scavenging activity. However, there was no effect on anthropometric indices and renal function based on BUN and creatinine. We also observed no significant hypolipidemic effect of saffron in our study.

In a meta-analysis by Pourmasoumi et al. [14], the efficacy of saffron supplementation on improving cardiovascular risk factors has been assessed in RCTs on patients with diabetes, metabolic syndrome, schizophrenia, major depressive disorder, coronary artery disease, and healthy subjects. The results of this meta-analysis

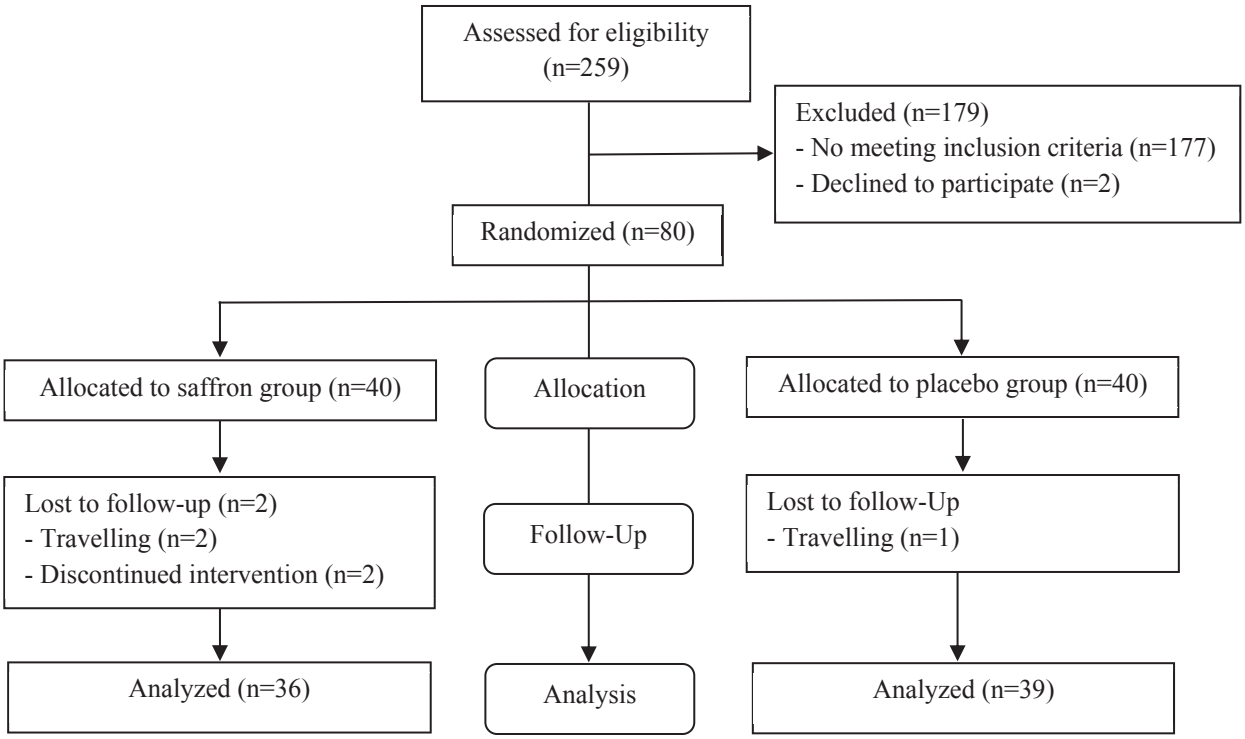

Fig. 1. Flowchart of patient enrollment for the randomized placebo-controlled trial of saffron supplementation in overweight/obese individuals with prediabetes.

did not show any benefit of saffron on blood lipids, as observed in our study. On the other hand, the authors reported a significant favorable effect on FBS and HbA1c, and also on some anthropometric measures including body weight and WC values. However, we failed to observe any significant effect of saffron intake on body composition, which may be related to the short follow-up period in our study.

In contrast to our findings, several animal studies have showed a significant reduction of blood lipids, and the prescribed dosage of saffron in these studies were between 25 and 100 mg/kg [43–46], which it is much higher compared with the dosage used in clinical trials. Therefore, it can be a reason for not observing any significant lipid-lowering effect of saffron in human studies. The effective mechanisms by which saffron reduce blood lipids in animal models involve the inhibition of the lymphatic absorption of cholesterol and the activity of pancreatic lipase as a competitive inhibitor [36].

Table 1
Demographic and anthropometric characteristics of participants at the baseline and at the end of the study.

Variable	Saffron (n = 36)	Placebo (n = 39)	*P* value[b]
Male/Female, %	35.1/64.9	35.6/64.4	0.94
Age, y	57.95 ± 8.12	57.9 ± 8.7	0.56
Physical activity (MET-h/week)			
Baseline	32.55 ± 1.71	32.54 ± 1.89	0.254
End	33.11 ± 2.45	33.17 ± 2.61	0.231
***P* value[a]**	0.867	0.645	
Weight (kg)			
Baseline	76.29 ± 3.46	74.51 ± 4.55	0.06
End	76.01 ± 3.40	74.14 ± 4.69	0.05
***P* value[a]**	0.01	<0.005	
BMI (kg/m^2)			
Baseline	29.35 ± 1.50	28.78 ± 2.02	0.17
End	29.24 ± 1.49	28.64 ± 1.99	0.14
***P* value[a]**	0.001	<0.001	
WC (cm)			
Baseline	95.65 ± 4.61	94.03 ± 6.42	0.213
End	95.29 ± 4.40	93.90 ± 6.34	0.272
***P* value[a]**	0.004	0.157	

Note. Data are expressed as mean ± standard deviation or frequency (%). *P* value [a]: within-group comparisons resulted from paired sample t-test. *P* value [b]: between-group comparisons from chi-square test for qualitative variables, and independent sample t-test for quantitative variables. MET; metabolic equivalent, BMI; body mass index, WC; waist circumference.

Table 2
Dietary intakes of participants at the baseline and at the end of the study.

Variable	Saffron (n = 36)	Placebo (n = 39)	*P* value[b]
Energy (kcal/d)			
Baseline	2167.87 ± 596.16	1938.83 ± 428.64	0.137
End	2146.29 ± 675.47	1876.17 ± 340.79	0.091
***P* value[a]**	0.79	0.34	
CHO (g/d)			
Baseline	303.35 ± 80.26	265.64 ± 59.52	0.075
End	297.61 ± 86.32	260.00 ± 45.23	0.070
***P* value[a]**	0.59	0.57	
Pro (g/d)			
Baseline	71.66 ± 30.83	58.34 ± 20.05	0.087
End	66.30 ± 25.24	55.51 ± 13.99	0.080
***P* value[a]**	0.101	0.256	
Fat (g/d)			
Baseline	75.36 ± 25.61	71.02 ± 16.49	0.495
End	76.33 ± 28.01	67.98 ± 13.20	0.201
***P* value[a]**	0.82	0.21	
CHO (% of energy)			
Baseline	56.07 ± 4.08	54.98 ± 2.96	0.157
End	55.93 ± 2.96	55.59 ± 2.35	0.716
***P* value[a]**	0.413	0.137	
Pro (% of energy)			
Baseline	12.90 ± 3.27	12.01 ± 2.87	0.272
End	12.30 ± 2.17	11.85 ± 1.82	0.421
***P* value[a]**	0.08	0.85	
Fat (% of energy)			
Baseline	31.03 ± 5.22	33.01 ± 2.32	0.121
End	31.77 ± 2.92	32.56 ± 1.75	0.258
***P* value[a]**	0.49	0.29	

Note. Data are expressed as mean ± standard deviation. *P* value [a]: within-group comparisons resulted from paired sample t-test. *P* value [b]: between-group comparisons resulted from independent sample t-test.

Table 3
Effects of saffron supplementation on serum levels of glycemic markers and lipid profile.

	Saffron group (n = 36)				Placebo group (n = 39)				*P* value[b]
	Week 0	Week 8	Change	*P* value[a]	Week 0	Week 8	Change	*P* value[a]	
FBS (mg/dL)	118.11 ± 3.55	109.14 ± 6.23	−7.97 ± 5.68	<0.005	119.15 ± 4.03	118.87 ± 6.27	−1.61 ± 6.79	0.76	<0.005
HbA1c (%)	5.85 ± 0.12	5.70 ± 0.11	−0.15 ± 0.11	<0.005	5.88 ± 0.11	5.92 ± 0.12	0.04 ± 0.09	0.01	<0.005
TC (mg/dL)	186.67 ± 17.22	184.54 ± 17.45	−2.13 ± 7.33	0.09	192.69 ± 13.57	190.88 ± 14.60	−1.81 ± 6.98	0.11	0.84
LDL-C (mg/dL)	114.75 ± 13.25	113.55 ± 12.77	−1.19 ± 6.08	0.25	120.31 ± 12.69	117.72 ± 11.34	−2.59 ± 6.42	0.01	0.34
HDL-C (mg/dL)	49.97 ± 11.62	50.25 ± 11.15	0.28 ± 2.32	0.48	52.20 ± 8.80	52.00 ± 9.80	−.20 ± 3.96	0.75	0.52
TG (mg/dL)	101.50 ± 20.34	100.22 ± 17.63	−1.27 ± 4.64	0.11	108.94 ± 18.20	107.84 ± 15.97	−1.10 ± 5.65	0.23	0.88

Note. Data are expressed as mean ± standard deviation. *P* value [a]: within-group comparisons resulted from paired sample t-test. *P* value [b]: between-group comparisons resulted from analysis of covariance in the adjusted models (adjusted for age, sex, and baseline values). FBS; fasting blood sugar, HbA1c; glycosylated hemoglobin, TC; total cholesterol, LDL; low density lipoprotein cholesterol, HDL; high density lipoprotein cholesterol, TG; triglyceride.

Table 4
Effects of saffron supplementation on serum levels of antioxidant and renal markers.

	Saffron group (n = 36)				Placebo group (n = 39)				*P* value[b]
	Week 0	Week 8	Change	*P* value[a]	Week 0	Week 8	Change	*P* value[a]	
BUN (mg/dL)	10.68 ± 2.92	10.55 ± 2.71	−0.13 ± 0.42	0.06	11.25 ± 3.30	11.11 ± 3.18	−0.14 ± 0.48	0.07	0.92
Cr (mg/dL)	1.07 ± 0.30	1.05 ± 0.28	−0.02 ± 0.05	0.06	1.10 ± 0.30	1.09 ± 0.31	−0.009 ± 0.02	0.057	0.39
DPPH (%)	11.06 ± 3.24	13.46 ± 3.33	2.40 ± 2.02	<0.005	10.00 ± 3.32	9.15 ± 2.94	−0.85 ± 2.11	0.01	<0.005

Note. Data are expressed as mean ± standard deviation. *P* value [a]: within-group comparisons resulted from paired sample t-test. *P* value [b]: between-group comparisons resulted from analysis of covariance in the adjusted models (adjusted for age, sex, and baseline values). BUN; blood urea nitrogen, Cr; creatinine, DPPH; diphenylpycrylhydrazyl.

However, lipid metabolism pathways in rat and mice models differ from humans, and so generalization of findings from animal studies to human beings should be done with caution.

As observed in our study, other clinical trials [14] or the animal studies [35,47,48] also showed the beneficial effect of saffron on glycemic control. There are some mechanisms that contributed to beneficial effect of saffron on glycemic control, such as inhibition of renal glucose reabsorption, reduction of insulin resistance by stimulation and regeneration of β cells in islets of Langerhans, and also enhancement of glucose uptake and insulin sensitivity in skeletal muscle cells mediated by adenosine monophosphate-activated protein kinase/acetyl-CoA carboxylase (AMPK/ACC) and mitogen-activated protein kinases (MAPKs) pathways [49,50].

According to the International Diabetes Federation (IDF) definition [51], HbA1c is measured to determine the average level of blood glucose over the past 2–3 months. Therefore, considering budget constraints and also possible less compliance and cooperation of patients in the longer follow-up period, we decided to perform this study for 2 months. Finally, our results showed that there was a statistically significant decrease in HbA1c levels after 2 months of saffron supplementation compared with placebo; however these changes were not clinically significant. It is likely that if HbA1c had been measured after a longer time period (3 months), there was more chance to observe clinically significant changes; however, due to mentioned reasons, it was not possible.

Another finding was increased levels of DPPH radical scavenging activity, which could be interpreted as the increased antioxidant activity is associated with the capacity of saffron to act as antioxidant by donating a hydrogen atom to the DPPH radical anion [25]. Saffron contains more than 150 volatile and several nonvolatile compounds, among them, crocins, picocrocin, crocetin, carotene and safranal are considered as major biologically active compounds [39,52]. These ingredients modulate the antioxidant gene expression and up-regulate mitochondrial antioxidant genes, which can result in a reduced generation of mitochondrial reactive oxygen radicals [50]. Another study by Mousavi et al. also reported that saffron extract and crocins could decrease the glucose-induced neurotoxicity and reactive oxygen radicals production [53].

As strength, our study was a double-blind randomized placebo-controlled trial with high compliance in consuming the saffron pills (90%). Moreover, assessment of dietary intake and physical activity level was considered in the study protocol. Given these strengths, some potential shortcomings should be considered in our study. First, this trial was designed for evaluating the effects of saffron supplementation at 2 months, and the changes observed after a 2-month intervention cannot necessarily be extrapolated to the long-term effects. Therefore, larger scale trials with longer follow-up are clearly needed to better establishing the long-term efficiency, safety and possible adverse effects. Second, serial assessments of outcomes were not conducted during the study period, and measured only at baseline and at the end of the follow-up period. Third, we did not stratify randomization by sex, which might possibly affect our results. However, the findings were not changed significantly after adjustments for sex. Moreover, compared with the prescribed dosages of saffron in animal studies, the chosen dose in our study may have been inadequate to induce significant changes in some variables, such as lipid profile.

In conclusion, 8 weeks of saffron supplementation in overweight/obese individuals with prediabetes could improve glycemic and antioxidant status with no effect on BUN and creatinine, and so it seems that saffron as a beneficial and relatively safe herbal supplement can be recommended for prediabetic individuals. However, this supplementation appeared did not affect lipid concentrations. Further large scale trials with longer follow-up period and higher doses are needed to better understand the efficiency and safety of saffron for long-term use as a routine treatment.

Conflict of interest

There was no conflict of interest for the authors of this article.

Statement of authorship

All authors were responsible for development of the study protocol. AN, RM and ASA initiated the idea of the study and designed the trial. EKN, AM and AN carried out the data collection. AN, RM, ASA and NRJ completed the data, performed statistical analyses and interpreted data. NRJ and EKN drafted the manuscript.

AN, ASA and SAM contributed to edit the manuscript. All authors approved the final manuscript.

Acknowledgements

We thank the Department of Medical Research, Shahid Sadoughi University of Medical Sciences (SSUMS) for funding this project. Special thanks to the patients who participated in this study.

References

[1] Ahwaide HS, Elbarghathi NM, Mohamed FF. Narrative review on role of vitamin D in type II diabetes and hyperlipidemia. 2018.
[2] NCD Risk Factor Collaboration. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4· 4 million participants. Lancet 2016;387:1513–30.
[3] Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract 2014;103:137–49.
[4] Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4–14.
[5] Juarez LD, Gonzalez JS, Agne AA, Kulczycki A, Pavela G, Carson AP, et al. Diabetes risk scores for hispanics living in the United States: a systematic review. Diabetes Res Clin Pract 2018;142:120–9.
[6] Zhang L, Zalewski A, Liu Y, Mazurek T, Cowan S, Martin JL, et al. Diabetes-induced oxidative stress and low-grade inflammation in porcine coronary arteries. Circulation 2003;108:472–8.
[7] Libby P, Plutzky J. Diabetic macrovascular disease: the glucose paradox? Circulation 2002;106:2760–3.
[8] Alexander CM, Landsman PB, Teutsch SM. Diabetes mellitus, impaired fasting glucose, atherosclerotic risk factors, and prevalence of coronary heart disease. Am J Cardiol 2000;86:897–902.
[9] American Diabetes Association. Standards of medical care in diabetes-2014. Diabetes Care 2014;37:S14–80.
[10] Zhang Y, Dall TM, Chen Y, Baldwin A, Yang W, Mann S, et al. Medical cost associated with prediabetes. Popul Health Manag 2009;12:157–63.
[11] Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med 2001;345:790–7.
[12] Mohamed SF, Mwangi M, Mutua MK, Kibachio J, Hussein A, Ndegwa Z, et al. Prevalence and factors associated with pre-diabetes and diabetes mellitus in Kenya: results from a national survey. BMC Public Health 2018;18:1215.
[13] Jafarnejad S, Keshavarz SA, Mahbubi S, Saremi S, Arab A, Abbasi S, et al. Effect of ginger (Zingiber officinale) on blood glucose and lipid concentrations in diabetic and hyperlipidemic subjects: a meta-analysis of randomized controlled trials. J Funct Foods 2017;29:127–34.
[14] Pourmasoumi M, Hadi A, Najafgholizadeh A, Kafeshani M, Sahebkar A. Clinical evidence on the effects of saffron (Crocus sativus L.) on cardiovascular risk factors: a systematic review meta-analysis. Pharmacol Res 2019;139:348–59.
[15] Kaseb F, Yazdanpanah Z, Biregani AN, Yazdi NB, Yazdanpanah Z. The effect of chamomile (Matricaria recutita L.) infusion on blood glucose, lipid profile and kidney function in Type 2 diabetic patients: a randomized clinical trial. Prog Nutr 2018;20:110–8.
[16] Rocha DMUP, Caldas APS, da Silva BP, Hermsdorff HHM, Alfenas RDCG. Effects of blueberry and cranberry consumption on type 2 diabetes glycemic control: a systematic review. Crit Rev Food Sci Nutr 2018:1–13.
[17] Shabani E, Sayemiri K, Mohammadpour M. The effect of garlic on lipid profile and glucose parameters in diabetic patients: a systematic review and meta-analysis. Prim Care Diab 2019;13:28–42.
[18] Milajerdi A, Jazayeri S, Bitarafan V, Hashemzadeh N, Shirzadi E, Derakhshan Z, et al. The effect of saffron (Crocus sativus L.) hydro-alcoholic extract on liver and renal functions in type 2 diabetic patients: a double-blinded randomized and placebo control trial. J Nutr Int Metab 2017;9:6–11.
[19] Azimi P, Ghiasvand R, Feizi A, Hariri M, Abbasi B. Effects of cinnamon, cardamom, saffron, and ginger consumption on markers of glycemic control, lipid profile, oxidative stress, and inflammation in type 2 diabetes patients. Rev Diabet Stud 2014;11:258–66.
[20] Azimi P, Ghiasvand R, Feizi A, Hosseinzadeh J, Bahreynian M, Hariri M, et al. Effect of cinnamon, cardamom, saffron and ginger consumption on blood pressure and a marker of endothelial function in patients with type 2 diabetes mellitus: a randomized controlled clinical trial. Blood Press 2016;25:133–40.
[21] Chang CL, Lin Y, Bartolome AP, Chen Y-C, Chiu S-C, Yang W-C. Herbal therapies for type 2 diabetes mellitus: chemistry, biology, and potential application of selected plants and compounds. Evid Based Complement Altern Med 2013;2013.
[22] Milajerdi A, Jazayeri S, Hashemzadeh N, Shirzadi E, Derakhshan Z, Djazayeri A, et al. The effect of saffron (Crocus sativus L.) hydroalcoholic extract on metabolic control in type 2 diabetes mellitus: a triple-blinded randomized clinical trial. J Res Med Sci 2018;23:16.
[23] Melnyk JP, Wang S, Marcone MF. Chemical and biological properties of the world's most expensive spice: Saffron. Food Res Int 2010;43:1981–9.
[24] Gismondi A, Serio M, Canuti L, Canini A. Biochemical, antioxidant and anti-neoplastic properties of Italian saffron (Crocus sativus L.). Am J Plant Sci 2012;3:1573.
[25] Assimopoulou A, Sinakos Z, Papageorgiou V. Radical scavenging activity of Crocus sativus L. extract and its bioactive constituents. Phytother Res Int J Dev Pharmacol Toxicol Eval Nat Prod Derivat 2005;19:997–1000.
[26] Chen Y, Zhang H, Tian X, Zhao C, Cai L, Liu Y, et al. Antioxidant potential of crocins and ethanol extracts of Gardenia jasminoides ELLIS and Crocus sativus L.: a relationship investigation between antioxidant activity and crocin contents. Food Chem 2008;109:484–92.
[27] Kanakis CD, Tarantilis PA, Tajmir-Riahi HA, Polissiou MG. Crocetin, dimethylcrocetin, and safranal bind human serum albumin: stability and antioxidative properties. J Agric Food Chem 2007;55:970–7.
[28] Tavakkol-Afshari J, Brook A, Mousavi SH. Study of cytotoxic and apoptogenic properties of saffron extract in human cancer cell lines. Food Chem Toxicol 2008;46:3443–7.
[29] Pitsikas N, Zisopoulou S, Tarantilis PA, Kanakis CD, Polissiou MG, Sakellaridis N. Effects of the active constituents of Crocus sativus L., crocins on recognition and spatial rats' memory. Behav Brain Res 2007;183:141–6.
[30] Ochiai T, Shimeno H, Mishima K-i, Iwasaki K, Fujiwara M, Tanaka H, et al. Protective effects of carotenoids from saffron on neuronal injury in vitro and in vivo. Biochim Biophys Acta Gen Subj 2007;1770:578–84.
[31] Hosseinzadeh H, Younesi HM. Antinociceptive and anti-inflammatory effects of Crocus sativus L. stigma and petal extracts in mice. BMC Pharmacol 2002;2:7.
[32] Khosravan V. Anticonvulsant effects of aqueous and ethanolic extracts of Crocus sativus L. stigmas in mice. Arch Iran Med 2002;5:44.
[33] Pitsikas N, Boultadakis A, Georgiadou G, Tarantilis P, Sakellaridis N. Effects of the active constituents of Crocus sativus L., crocins, in an animal model of anxiety. Phytomedicine 2008;15:1135–9.
[34] Akhoundzadeh S, Falahpour H, Afkham K, Jamshidi A, KHALIGHI CF, Miller L. A comparative trial of Crocus sativus L.(saffron) and imipramine in mild to moderate depression. 2005.
[35] 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. J Nutr Biochem 2007;18:64–72.
[36] Sheng L, Qian Z, Zheng S, Xi L. Mechanism of hypolipidemic effect of crocin in rats: crocin inhibits pancreatic lipase. Eur J Pharmacol 2006;543:116–22.
[37] Xi L, Qian Z, Shen X, Wen N, Zhang Y. Crocetin prevents dexamethasone-induced insulin resistance in rats. Planta Med 2005;71:917–22.
[38] Vakili S, Savardashtaki A, Moghaddam MAM, Nowrouzi P, Shirazi MK, Ebrahimi G. The effects of saffron consumption on lipid profile, liver enzymes, and oxidative stress in male hamsters with high fat diet. Trends Pharm Sci 2017;3:201–8.
[39] Hosseinzadeh H, Sadeghnia HR, Ghaeni FA, Motamedshariaty VS, Mohajeri SA. Effects of saffron (Crocus sativus L.) and its active constituent, crocin, on recognition and spatial memory after chronic cerebral hypoperfusion in rats. Phytother Res 2012;26:381–6.
[40] Tabeshpour J, Sobhani F, Sadjadi SA, Hosseinzadeh H, Mohajeri SA, Rajabi O, et al. A double-blind, randomized, placebo-controlled trial of saffron stigma (Crocus sativus L.) in mothers suffering from mild-to-moderate postpartum depression. Phytomedicine 2017;36:145–52.
[41] Moghaddam MB, Aghdam FB, Jafarabadi MA, Allahverdipour H, Nikookheslat SD, Safarpour S. The Iranian Version of International Physical Activity Questionnaire (IPAQ) in Iran: content and construct validity, factor structure, internal consistency and stability. World Appl Sci 2012;18:1073–80.
[42] Janaszewska A, Bartosz G. Assay of total antioxidant capacity: comparison of four methods as applied to human blood plasma. Scand J Clin Lab Invest 2002;62:231–6.
[43] Lari P, Rashedinia M, Abnous K, Hosseinzadeh H. Crocin improves lipid dysregulation in subacute diazinon exposure through ERK1/2 pathway in rat liver. Drug Res 2014;64:301–5.
[44] Mashmoul M, Azlan A, Yusof BNM, Khaza'ai H, Mohtarrudin N, Boroushaki MT. Effects of saffron extract and crocin on anthropometrical, nutritional and lipid profile parameters of rats fed a high fat diet. J Funct Foods 2014;8:180–7.
[45] Shirali S, Zahra Bathaie S, Nakhjavani M. Effect of crocin on the insulin resistance and lipid profile of streptozotocin-induced diabetic rats. Phytother Res 2013;27:1042–7.
[46] Samarghandian S, Azimi-Nezhad M, Farkhondeh T. Immunomodulatory and antioxidant effects of saffron aqueous extract (Crocus sativus L.) on streptozotocin-induced diabetes in rats. Indian Heart J 2017;69:151–9.
[47] Mohajeri D, Mousavi G, Doustar Y. Antihyperglycemic and pancreas-protective effects of Crocus sativus L.(Saffron) stigma ethanolic extract on rats with alloxan-induced diabetes. J Biol Sci 2009;9:302–10.
[48] Elgazar AF, Rezq AA, Bukhari HM. Anti-hyperglycemic effect of saffron extract in alloxan-induced diabetic rats. Eur J Biol Sci 2013;5:14–22.
[49] Kang C, Lee H, Jung E-S, Seyedian R, Jo M, Kim J, et al. Saffron (Crocus sativus L.) increases glucose uptake and insulin sensitivity in muscle cells via multi-pathway mechanisms. Food Chem 2012;135:2350–8.

[50] Farkhondeh T, Samarghandian S. The effect of saffron (Crocus sativus L.) and its ingredients on the management of diabetes mellitus and dislipidemia. Afr J Pharm Pharmacol 2014;8:541–9.
[51] Diabetes glossary, glycosylated haemoglobinA1c (HbA1c). international diabetes federation; June 25, 2019. on the World Wide Web: https://www.idf.org/aboutdiabetes/glossary.html.
[52] Rios J, Recio M, Giner R, Manez S. An update review of saffron and its active constituents. Phytother Res 1996;10:189–93.
[53] Mousavi SH, Tayarani N, Parsaee H. Protective effect of saffron extract and crocin on reactive oxygen species-mediated high glucose-induced toxicity in PC12 cells. Cell Mol Neurobiol 2010;30:185–91.