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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

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ABSTRACT

Background and aim: Uncontrolled diabetes causes liver and renal dysfunctions. Since, saffron may improve diabetes control and indicate renal and liver protection, this study purposed to illustrate for the first time the effects of saffron extract on some liver and renal functional parameters among diabetic patients.

Materials and methods: In this double-blind clinical trial, 54 type 2 diabetic patients were randomly recruited to consume either 15 mg saffron extract (n = 27) or placebo capsules (n = 27) twice a day for 8 weeks. Alkaline phosphatase, aspartate and alanine amino transferase, uric acid, blood urea nitrogen, and creatinine of the patients as well as their physical activity, dietary intakes, anthropometric measures and blood pressure were measured. Data were analyzed by SPSS.18 software.

Results: Uric acid and blood urea nitrogen were significantly decreased in the saffron group ($P < 0.05$), however, there were no significant differences between the two groups at the end of the study ($p = 0.29$ and 0.14, respectively). Moreover, changes in other profiles, including liver enzymes, were not statistically significant in the two groups. Also, no significant changes in blood pressure, dietary intakes, and physical activity were seen among the two groups.

Conclusion: Saffron hydro-alcoholic extract did not considerably improve renal and liver functions in T2DM patients in an 8-week randomized clinical trials. The results deserved further investigations with more accurate methods to confirm.

1. Introduction

Type 2 Diabetes Mellitus (T2DM) is a chronic costly public health problem which its prevalence is increasing worldwide [1,2]. In addition, recent evidences have shown a dramatic increase in the prevalence of diabetes in Iran, during the last decade [3]. Poorly controlled diabetes is associated with some complications including atherosclerosis, retinopathy, nephropathy, and neuropathy [4,5]. Because of hyperglycemia, liver diseases including fatty liver disease are also very prevalent among diabetic patients [5]. Clinically, Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), and Alkaline Phosphatase (ALP) enzymes are commonly measured to determine the severity of liver damages [6–8]. In addition, due to the deleterious effects of hyperglycemia on renal function, a considerable number of diabetic patients also

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require dialysis [9].

According to the mentioned complications, blood glucose control in diabetes is very important. Life style and diet changes may improve diabetes control [10]. In addition, conventional drugs may also control blood glucose in short term. However, side effects of these medicines and inconclusive treatment have taken the attentions to new and complementary treatments, including herbal medicine [11,12]. Dried stigma of saffron (*Crocus sativus* L.) is an herbal medicine in Islamic-Persian traditional medicine. Saffron is a native plant in Iran which has been used as the most expensive traditional spice for many years [13–15]. Therapeutic values of saffron are contributed to its active constituents, including crocin, safranal, crocetin, and picrocrocin. Flavonoids and carotenoids are also found in the saffron extract [16,17]. Bandegi et al. have demonstrated that 30 mg/kg daily injection of saffron or crocin at similar doses for 21 days reduce oxidative stress in the animal liver, kidney, and brain [18]. In addition, a recent study have shown that saffron and its crocin dose-dependently alleviated levels of liver enzymes in male rats suffered from fatty liver disease [19]. Previous studies were also shown that saffron may protect kidney and liver from environmental toxins [20,21].

To the best of our knowledge, there is no separate human study about the effect of saffron hydro-alcoholic extract on liver and kidney function parameters in T2DM patients who are prone to the liver and renal dysfunctions. Beside the results from animal model studies, saffron may improve renal and liver protection because of its anti-inflammatory and antioxidant properties. In addition, saffron may independently alleviate diabetes, and by this way reduce diabetes complications. Given these reasons, current clinical trial aimed to study the effect of hydro-alcoholic extract of saffron (*Crocus sativus* L.) on the parameters of liver and renal functions in T2DM patients.

2. Materials and methods

2.1. Participants

This study was an 8-weeks randomized and double-blind clinical trial. The study was done on outpatients of Natanz Diabetes Society, Isfahan, Iran, between September 2014 and May 2015. The study sample size was calculated using standard formula for clinical trials by considering type I (a) and type II errors (b) as 0.05 and 0.20 (study power = 80%), respectively, and ALT as key variable. We predicted 30% reduction in ALT concentration due to the intervention [19].

Among all registered patients in the society, 54 T2DM patients were selected according to the inclusion criteria: suffering from type 2 diabetes mellitus (fasting blood sugar≥ 126 mg/dL), 40 to 65 years old, controlled diabetes (fasting blood glucose< 170 mg/dl), Body Mass Index (BMI) 18.5–30 kg/m^2. Subjects were excluded if: being less than 40 and more than 65 years old, consuming other drugs except blood glucose controlling drugs or higher than determined doses (up to 1.5 gr metformin, or 10 mg glibenclamide), tobacco or opium use, insulin injection, uncontrolled blood glucose (≥170 mg/dl), pregnancy or lactating or patients were preparing for pregnancy, other diseases than diabetes, BMI less than 18.5 or more than 40 kg/m^2.

Study protocol and the consent form were approved by the Tehran University of Medical Sciences Ethics committee (ir.tums.rec.1394.9211468004–143703; research.tums.ac.ir). The trial has registered at Iranian Registry of Clinical Trials (IRCT2015082623776N1; www.irct.ir).

2.2. Study design

Before intervention, participants were explained about study aims and details, then were asked to sign an informed consent form.

Participants were stratified based on sex (male/female) and age (<50 or ≥50 years), then randomly divided into two similar groups (n = 27) to receive either placebo or saffron capsules twice a day for 8 weeks. This deviation was blinded from all of the project performers, participants, and analyzers until end of data analysis and conducted by the physician of the society. Randomization was performed using computer-generated random numbers.

In this study we used Safrotin as a commercially available saffron capsule containing concentrated hydro-alcoholic stigma extract of saffron. Each capsules contained 15 mg of placebo or saffron hydro-alcoholic extract which was standardized by crocin. Each capsule contains starch, lactose, magnesium stearate, and gelatin. Safrotin capsules were donated by Green Plants of Life Co. (IMPIRAN; Tehran, Iran). Placebo capsules were similar to the saffron capsules in appearance, color, and size. Placebo capsules also contained starch, lactose, magnesium stearate, and gelatin as well as saffron essence. Placebo and saffron capsules were labeled as 1 and 2, respectively, by IMPIRAN Co.

Participants were asked not to change their diet, physical activity, and drugs during the intervention. They were also asked to refer every 2 weeks (2nd, 4th, 6th, and 8th weeks) to receive their capsules. To assessing their compliance, participants brought their capsule's boxes in every periodical visits to determining total consumed capsules.

At the beginning of study, 4th week, and the end of study three 24-h dietary recalls were taken from participants, by a skilled nutritionist. Dietary intakes of macronutrients and total energy were determined using the Nutritionist IV (N- IV) software, modified for Iranian food items. To assess physical activity, participants completed the International Physical Activity Questionnaire (IPAQ) at the beginning, during each periodical visits, and the end of study. Physical activity measured by the IPAQ were then converted to Metabolic Equivalents (METs) [22,23].

2.3. Assessment of exposure

At the study beginning, participants were referred to the central laboratory of Natanz, Isfahan, Iran. After 12 h fasting, patients' blood samples were recruited, then, their serum was separated and kept at −70 °C. ALT, AST, ALP, Creatinine (Cr), Blood Urea Nitrogen (BUN), and uric acid concentrations were measured using calorimetric method by commercial kit (Pars Azmoon Co., Tehran, Iran). After 8 weeks intervention, these examinations were repeated.

2.4. Assessment of other variables

Participants' weight, height, Waist circumference (WC), and blood pressure were measured at the study beginning. Weight measured using a digital scale (Sega 707, Hamburg, Germany) with the nearest of 100 g without shoes and with light clothes, height by the use of a stadiometer (Seca, Hamburg, Germany) without shoes to the nearest of 0.1 cm, and WC at the narrowest level using a non-stretchable tape to the nearest of 0.1 cm. Body Mass Index (BMI) was calculated by deviation of weight (kg) to squared height (cm^2). Systolic and diastolic blood pressure were measured twice, from the right arm of participants who were sitting for at least 10 min. Blood pressure was measured using a standard barometer which was calibrated by the Institute of Standard and Industrial Research of Iran.

During the periodical visits, participants were assessed by the

Table 1
Differences in the basic characteristics between the saffron and placebo groups.

Variables		Saffron Group	Placebo Group	*P Value*[c]
Age, Year (mean ± SD)		54.57 ± 6.96	55.42 ± 7.58	0.67*
BMI,[a] Kg/Cm^2 (mean ± SD)		23.84 ± 11.89	28.30 ± 3.24	0.29*
Weight, Kg (mean ± SD)		63.10 ± 31.64	66.34 ± 9.01	0.71*
WC,[b] *Cm* (mean ± SD)		98.25 ± 10.08	96.51 ± 8.13	0.60*
Family Numbers (mean ± SD)		3.46 ± 1.27	3.00 ± 1.25	0.19*
Sex (n)	Male	6	6	1.00*
	Female	12	12	
Job (n)	Workless	0	0	0.18**
	Employee	0	1	
	Self-Employment	1	3	
	Farmer	0	0	
	Housewife	18	20	
	Retired	7	2	
	Disabled	0	0	
Drug (n)	Metformin	9	5	0.40**
	Metformin + Glibenclamide	7	7	
	Glibenclamide	10	14	
Marriage	Single	0	0	0.68**
	Married	22	23	
	(n) Widow	4	3	
	Divorced	0	0	
Education	Illiterate	2	9	0.16**
	Primary Education	14	13	
	(n) High School Diploma	6	3	
	University Degree	4	1	
Income (n)	Low	11	16	0.22**
	Medium	15	9	
	High	0	1	

[a] Body Mass Index.
[b] Waist circumference.
[c] P. Value for variable comparing between the two groups (P < 0.05). Calculated by Independent T Test (*) and Fisher exact test (**).

psychiatrist and their weight, height, WC, and blood pressure were measured. Moreover, side effects of the capsules were recorded non-systemically by asking from the patients. At the end of study, weight, height, WC, and blood pressure were measured again.

2.5. Statistical analysis

Normal distribution of data was investigated using Kolmogorov–Smirnov. Data was expressed as mean ± standard deviation. To compare basal characteristics and physical activity (METs) between the two groups, Independent-Samples T test was used. Fisher exact test was used to compare basal qualitative characteristics between groups. Laboratory results were compared within groups using Paired-Samples T test. ANOVA analysis of covariance after adjustment for basal results was used to compare final results between groups. Baseline dietary intakes were compared between groups by Independent-Samples T Test. Time effect on dietary intakes was determined using repeated measure analysis of variance.

P < 0.05 was considered statistically significant. All statistical analyses were done using the statistical package for social sciences (SPSS) for Windows version 18 (SPSS Inc., Chicago, IL).

3. Results

Basic characteristics of the participants are shown in Table 1. There was no significant difference between the two groups with regard to the basic characteristics.

Fifty two participants completed the study. One participant from the placebo and one participant from the saffron group voluntarily existed the study at the midst of intervention.

Independent T test did not show any significant differences at dietary intakes between the two groups at the study beginning (Table 2). Moreover, differences of dietary intakes within groups was also non-significant (Fig. 1).

In addition, differences of physical activity (based on METs) between the two groups at the study beginning were non-significantly (P = 0.09). Participant's physical activity did not also change significantly within groups (P = 0.87 and 0.44, respectively) (Table 3).

Differences of BUN, Uric acid, AST, ALT, and ALP between groups were non-significant at the study beginning as well as the end (Table 3). Although, BUN and Uric acid were significantly decreased in the saffron group (P = 0.01 and 0.02, respectively), decreases in AST and ALT were non-significant (P = 0.96 and 0.67, respectively). In addition, increases in ALP was also non-significant in the saffron group (P = 0.15). Creatinine concentration did not change

Table 2
Differences in the dietary intakes intra and inter the saffron and placebo groups.

Variables	Saffron group (mean ± SD)	Placebo group (mean ± SD)	*P. Value*
Energy (C)	1363.40 ± 424.07	1488.80 ± 503.70	0.35
Total Carbohydrate (gr)	216.60 ± 74.85	237.75 ± 92.89	0.38
Total Protein (gr)	56.67 ± 18.52	58.91 ± 20.97	0.69
Total Fat (gr)	35.29 ± 16.22	36.93 ± 21.69	0.76

*P. Value for variable comparing between the two groups at the beginning of the intervention. Calculated by Independent T Test.

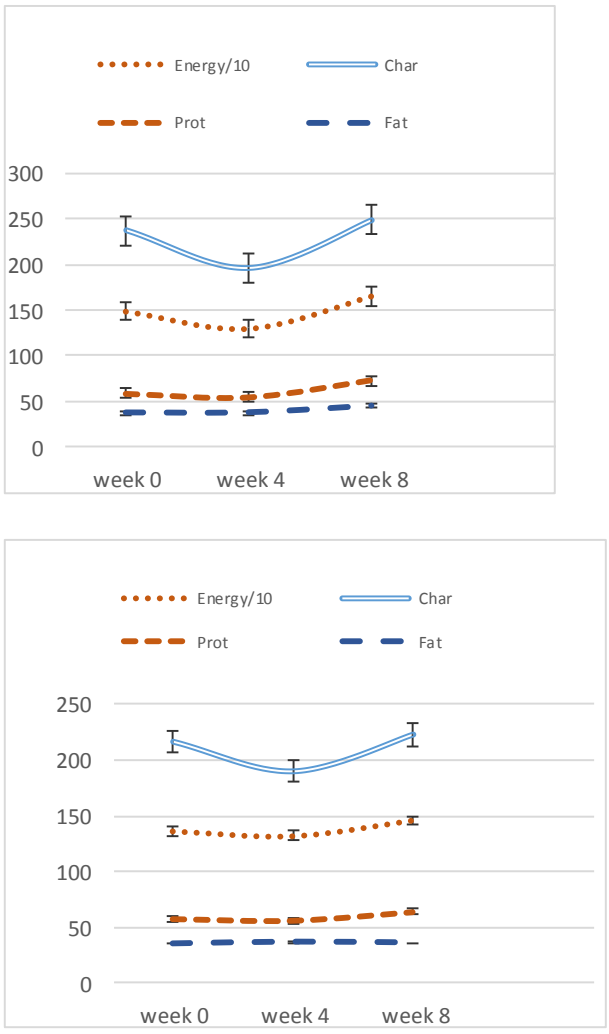

Fig. 1. Changes in the dietary intakes during the intervention at the saffron group (right) and the placebo group (left) (P > 0.05).

significantly during intervention at the saffron group (P = 1.00). Furthermore, decreases in uric acid, BUN, and AST, and increases in creatinine, ALT, and ALP concentrations were non-significant at the placebo group. (Table 3).

Although, systolic and diastolic blood pressures increased in the saffron group at the end of study compared to the beginning (average 14.69 and 8.63 mmHg, respectively), these changes were non-significant (P = 0.68 and 0.86, respectively). In addition, changes in systolic and diastolic blood pressures were non-significant in the placebo group (P = 0.39 and 0.81, respectively). Furthermore, anthropometric measures did not also change significantly within the two groups (Fig. 2).

4. Discussion

The study results showed that the saffron could not decreas BUN,uric acid, and cretinine in diabetic patients, which are susceptible for renal diseases, compared to placebo. However, BUN and uric acid reductions were significant within the saffron group. Although, Liver enzymes and ALK were decreased at the saffron group, this reduction was not significant. Although, the effect of saffron extract on liver and renal parameters are rarely investigated at the available studies, a new animal model study has shown BUN and Cr significant reduction in the presence of crocin in diabetic rats. Since, oxidative stress reduction has been shown in this study, the authors have attributed kidney protective effects of crocin to oxidative stress reduction [21]. Two other similar animal studies have also shown kidney protective effects of saffron and its crocin in rats with kidney damages [24,25]. Bandegi et al. study has found that daily injection of 30 mg/kg saffron or crocin at similar dose for 21 days, reduces chronic stress-induced oxidative stress in rats kidney, liver, and brain [18]. Based on the Omidi et al. study, Liver protective effects of saffron have been demonstrated in Wistar rats at acetaminophen-induced liver stress. In the present study, saffron has reduced ALT and AST at a dose of 20 mg/kg [20]. It has been also found that saffron may attenuate glucose absorption enzymes and in turn improve diabetes control [26]. In addition, antioxidant capacity of saffron is very considerable as compared to other spices [13,26,27].

As the changes of blood pressure indices and anthropometric measures were non significant during the study, it seems that the results are not related to blood pressure control or anthropometric changes.

Physical activity and dietary intake of the participants have not also changed significantly during the study. So, non-significant renal parameters and liver enzymes reduction in the saffron group compared with the placebo group is not in relation with physical activity or dietary intakes changes and may be attributed to the study short duration.

Table 3
Laboratory indicators and physical activity levels inter and intra the saffron and placebo groups.

Variables	Saffron group			Placebo group			Bef. Int[a]	Aft. Int[b]
	Bef Int[a] (mean ± SD)	Aft Int[b] (mean ± SD)	P_a	Bef Int[a] (mean ± SD)	Aft Int[b] (mean ± SD)	P_a	P_b	P_c
BUN[c]	28.94 ± 5.59	24.47 ± 8.10	0.01	30.56 ± 2.21	28.60 ± 9.68	0.16	0.37	0.14
Creatinine	0.76 ± 0.18	0.76 ± 0.12	1	0.78 ± 0.14	0.80 ± 0.14	0.30	0.59	0.34
Uric Acid	4.80 ± 0.28	4.18 ± 0.77	0.02	4.86 ± 0.99	4.55 ± 1.15	0.43	0.67	0.29
AST[d]	22.26 ± 7.62	23.21 ± 7.18	0.96	25.70 ± 9.49	24.90 ± 9.76	0.55	0.33	0.53
ALT[e]	28.21 ± 11.66	27.21 ± 9.60	0.67	24.75 ± 10.59	25.45 ± 11.99	0.63	0.30	0.81
ALP[f]	170.69 ± 38.95	185.69 ± 48.17	0.15	188.39 ± 36.50	191.28 ± 30.42	0.70	0.09	0.66
METs[g]	1193.80 ± 621.21	1181.50 ± 555.17	0.87	942.33 ± 435.49	872.89 ± 341.42	0.44	0.09	0.10

P_a: P. Value for variable changes during the intervention intra group. Calculated by Paired-Samples T Test.
P_b: P. Value for variable comparing between the two groups at the beginning of the intervention. Calculated by Independent T Test.
P_c: P. Value for variable comparing between the two groups at the end of the intervention after adjusting for basal values. Calculated by ANOVA analysis of covariance.
[a] Before Intervention.
[b] After Intervention.
[c] Blood Urea Nitrogen.
[d] Aspartate Amino Transferase.
[e] Alanine Amino Transferase.
[f] Alkaline Phosphatase.
[g] Metabolic Equivalents.

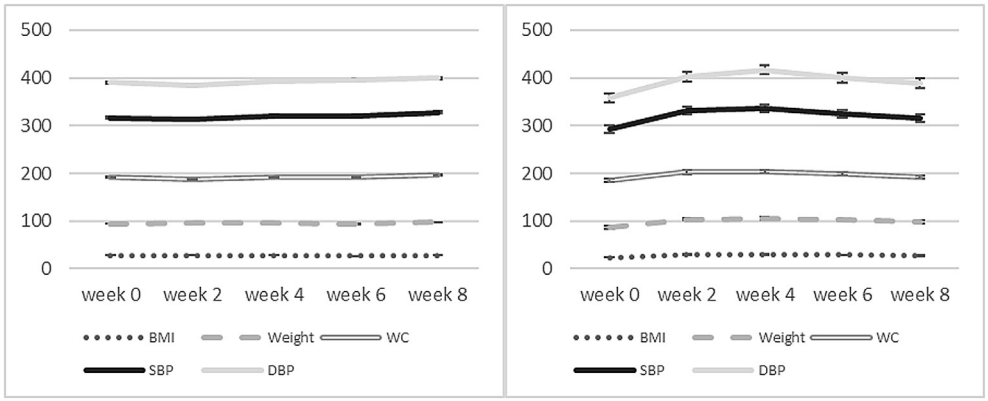

Fig. 2. Anthropometric measurements and blood pressure parameters changes during the study at the saffron (right) and placebo (left) groups * Calculated by ANOVA repeated measure (P > 0.05) BMI: Body Mass Index WC: Waist Circumference SBP: Systolic Blood Pressure DBP: Diastolic Blood Pressure.

In addition, saffron is a safe spice with the minimum toxicity on the body normal cells [28]. However, to determine saffron effects on renal and kidney functions, long term clinical trials with larger populations are recommended for future researches. Moreover, it is necessary to use more accurate clinical tests to assess liver and kidney functions.

In conclusion, although, saffron hydro-alcoholic extract may improve renal protection in saffron treated type 2 diabetic patients by itself, the protective effect was not significant when compared with placebo. Saffron effects on liver function were not also significant in this intervention compared with placebo. It should be noted that this study is the first study investigating the effect of saffron extract on liver and kidney functions among type 2 diabetic patients, therefore, further studies are needed to confirm these findings.

Conflict of interest

All of The authors declare that there was no conflict of interest.

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References

[1] Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, et al. Heart disease and stroke statistics-2014 update. Circulation 2014;129(3).
[2] Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss an update of the 1997 American Heart Association Scientific statement on obesity and heart disease from the obesity committee of the council on nutrition, physical activity, and metabolism. Circulation 2006;113(6): 898–918.
[3] Jafarnejad S, Saremi S, Jafarnejad F, Arab A. Effects of a multispecies probiotic mixture on glycemic control and inflammatory status in women with gestational diabetes: a randomized controlled clinical trial. J Nutr Metab 2016;2016. 5190846.
[4] Horwich TB, Fonarow GC, Hamilton MA, MacLellan WR, Woo MA, Tillisch JH. The relationship between obesity and mortality in patients with heart failure. J Am Coll Cardiol 2001;38(3):789–95.
[5] Lavie CJ, Osman AF, Milani RV, Mehra MR. Body composition and prognosis in chronic systolic heart failure: the obesity paradox. Am J Cardiol 2003;91(7): 891–4.
[6] Vicariotto F, Mogna L, Del Piano M. Effectiveness of the two microorganisms Lactobacillus fermentum LF15 and Lactobacillus plantarum LP01, formulated in slow-release vaginal tablets, in women affected by bacterial vaginosis: a pilot study. J Clin Gastroenterol 2014;48(1):106–12.
[7] Jiang XR, Awati A, Agazzi A, Vitari F, Ferrari A, Bento H, et al. Effects of a blend of essential oils and an enzyme combination on nutrient digestibility, ileum histology and expression of inflammatory mediators in weaned piglets. Animal 2015;9(3):417–26.
[8] Szatraj K, Szczepankowska AK, Saczynska V, Florys K, Gromadzka B, Lepek K, et al. Expression of avian influenza haemagglutinin (H5) and chicken interleukin 2 (chIL-2) under control of the ptcB promoter in Lactococcus lactis. Acta Biochim Pol 2014;61(3):609–14.
[9] Nabatanzi R, Bayigga L, Ssinabulya I, Kiragga A, Kambugu A, Olobo J, et al. Low antigen-specific CD4 T-cell immune responses despite normal absolute CD4 counts after long-term antiretroviral therapy an African cohort. Immunol Lett 2014;162(2):264–72.
[10] Hunking P. Lifestyle advice in preventing and treating type 2 diabetes. J Fam Health 2005;16(4):109–12.
[11] Inoue Y, Kambara T, Murata N, Komori-Yamaguchi J, Matsukura S, Takahashi Y, et al. Effects of oral administration of Lactobacillus acidophilus L-92 on the symptoms and serum cytokines of atopic dermatitis in Japanese adults: a double-blind, randomized, clinical trial. Int Arch Allergy Immunol 2014;165(4):247–54.
[12] Vitaglione P, Mennella I, Ferracane R, Rivellese AA, Giacco R, Ercolini D, et al. Whole-grain wheat consumption reduces inflammation in a randomized controlled trial on overweight and obese subjects with unhealthy dietary and lifestyle behaviors: role of polyphenols bound to cereal dietary fiber. Am J Clin Nutr 2015;101(2):251–61.
[13] Abdullaev F, Espinosa-Aguirre J. Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials. Cancer Detect Prev 2004;28(6):426–32.
[14] Akhondzadeh S, Sabet MS, Harirchian M, Togha M, Cheraghmakani H, Razeghi S, et al. Saffron in the treatment of patients with mild to moderate Alzheimer's disease: a 16-week, randomized and placebo-controlled trial. J Clin Pharm Ther 2010;35(5):581–8.
[15] Xing Y, Wang S, Fan J, Oso AO, Kim SW, Xiao D, et al. Effects of dietary supplementation with lysine-yielding Bacillus subtilis on gut morphology, cecal microflora, and intestinal immune response of Linwu ducks. J Anim Sci 2015;93(7):3449–57.
[16] Sepideh A, Karim P, Hossein A, Leila R, Hamdollah M, Mohammad EG, et al. Effects of multistrain probiotic supplementation on glycemic and inflammatory indices in patients with nonalcoholic fatty liver disease: a double-blind randomized clinical trial. J Am Coll Nutr 2015;2:1–6.
[17] 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(12):1135–9.
[18] Kwon HH, Yoon JY, Park SY, Min S, Suh DH. Comparison of clinical and histological effects between lactobacillus-fermented Chamaecyparis obtusa and tea tree oil for the treatment of acne: an eight-week double-blind randomized controlled split-face study. Dermatology 2014;229(2):102–9.
[19] Mashmoul M, Azlan A, Mohtarrudin N, Yusof BNM, Khaza'ai H, Khoo HE, et al. Protective effects of saffron extract and crocin supplementation on fatty liver tissue of high-fat diet-induced obese rats. BMC Complement Altern Med 2016;16(1):401.
[20] Tajadadi-Ebrahimi M, Bahmani F, Shakeri H, Hadaegh H, Hijijafari M, Abedi F, et al. Effects of daily consumption of synbiotic bread on insulin metabolism and serum high-sensitivity C-reactive protein among diabetic patients: a double-blind, randomized, controlled clinical trial. Ann Nutr Metab 2014;65(1):34–41.

[21] Huang HY, Chen YC, Wang PC, Tsai MA, Yeh SC, Liang HJ, et al. Efficacy of a formalin-inactivated vaccine against Streptococcus iniae infection in the farmed grouper Epinephelus coioides by intraperitoneal immunization. Vaccine 2014;32(51):7014–20.
[22] Harslof LB, Damsgaard CT, Andersen AD, Aakjaer DL, Michaelsen KF, Hellgren LI, et al. Reduced ex vivo stimulated IL-6 response in infants randomized to fish oil from 9 to 18 months, especially among PPARG2 and COX2 wild types. Prostagl Leukot Essent Fat Acids 2015;94:21–7.
[23] Villar-Garcia J, Hernandez JJ, Guerri-Fernandez R, Gonzalez A, Lerma E, Guelar A, et al. Effect of probiotics (Saccharomyces boulardii) on microbial translocation and inflammation in HIV-treated patients: a double-blind, randomized, placebo-controlled trial. J Acquir Immune Defic Syndr 2015;68(3):256–63.
[24] Yang OO, Kelesidis T, Cordova R, Khanlou H. Immunomodulation of antiretroviral drug-suppressed chronic HIV-1 infection in an oral probiotic double-blind placebo-controlled trial. AIDS Res Hum Retroviruses 2014;30(10):988–95.
[25] Jensen GS, Carter SG, Reeves SG, Robinson LE, Benson KF. Anti-inflammatory properties of a dried fermentate in vitro and in vivo. J Med food 2015;18(3):378–84.
[26] Loizzo MR, Marrelli M, Pugliese A, Conforti F, Nadjafi F, Menichini F, et al. Crocus cancellatus subsp. damascenus stigmas: chemical profile, and inhibition of α-amylase, α-glucosidase and lipase, key enzymes related to type 2 diabetes and obesity. J Enzyme Inhib Med Chem 2016;31(2):212–8.
[27] Milajerdi A, Bitarafan V, Mahmoudi M. A review on the effects of saffron extract and its constituents on factors related to neurologic, cardiovascular and gastrointestinal diseases. J Med Plants 2015;3(55):9–28.
[28] Milajerdi A, Djafarian K, Hosseini B. The toxicity of saffron (Crocus sativus L.) and its constituents against normal and cancer cells. J Nutr Intermed Metab 2016;3:23–32.