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Effect of *Ziziphus jujube* Fruit Infusion on Lipid Profiles, Glycaemic Index and Antioxidant Status in Type 2 Diabetic Patients: A Randomized Controlled Clinical Trial

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This study was designed to assess the effects of *Ziziphus jujube* fruit (ZJF) infusion on lipid profiles, glycaemic control and antioxidant status in patients with type 2 diabetes mellitus (T2DM). In this randomized controlled clinical trial, 116 participants with T2DM (older than 30 years) were assigned to consume a balanced diet or diet plus ZJF infusion (10 g/100 mL boiling water) three times/day before main meals for 12 weeks. Diet was designed to be energy restricted (500 kcal/day deficit from estimated energy requirements), and macronutrient content was similar in both groups (55% carbohydrate, 15% protein and 30% fat). The consumption of ZJF infusion compared with the control group was associated with significant improvement in glycosylated haemoglobin (−0.68 ± 0.65 vs. −0.44 ± 0.55%; p = 0.03), total cholesterol (−24.29 ± 28.89 vs. −11.21 ± 29.98 mg/dL; p = 0.02), triglycerides (−43.3 ± 39.26 vs. −27.16 ± 46.84 mg/dL; p = 0.05), low-density lipoprotein cholesterol (−19.85 ± 27.62 vs. −6.55 ± 27.82 mg/dL; p = 0.01), low-density lipoprotein cholesterol/high-density lipoprotein cholesterol (−0.56 ± 0.80 vs. −0.2 ± 0.72; p = 0.01) and total cholesterol to high-density lipoprotein cholesterol ratios (−0.73 ± 0.94 vs. −0.35 ± 0.77; p = 0.02). ZJF had beneficial effects on glycosylated haemoglobin and lipid profile in T2DM patients. Further research is needed to identify the mechanism of ZJF action on glucose and lipid metabolism.

Keywords: type 2 diabetes mellitus; *Ziziphus jujube*; glycaemic index; lipid profile; antioxidant status.

Abbreviations Used: 2hpp, 2 h postprandial glucose; FBS, fasting blood sugar; HbA1c, glycosylated haemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LDL-C/HDL-C ratio, low-density lipoprotein cholesterol to high-density lipoprotein cholesterol ratio; MDA, malondialdehyde; T2DM, type 2 diabetes mellitus; TAC, total antioxidant capacity; TC, total cholesterol; TC/HDL-C ratio, total cholesterol to high-density lipoprotein cholesterol ratio; TG, triglycerides; VLDL-C, very low-density lipoprotein cholesterol; ZJF, *Ziziphus jujube* fruit

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is considered as a common metabolic disorder characterized by chronic high blood glucose level, which results from insulin secretory dysfunction and/or defects in insulin action. Chronic hyperglycaemia is associated with some complications, especially neuropathy, nephropathy, retinopathy and cardiovascular disease (Nathan, 1993). The World Health Organization (WHO) stated that 9.5% of the adult population in the world (approximately 347 million people) were living with diabetes in 2008. It is estimated that this number will reach 552 million by 2030, and the greatest increase in prevalence of diabetes will occur in Asia, the Middle East and North Africa region (Zhao *et al.*, 2014).

Various strategies, including the use of oral hypoglycaemic agents and insulin therapy, have been used for alleviating hyperglycaemia. In spite of the use of modern synthetic treatments, there is an increasing interest in the use of complementary and alternative approaches such as traditional medicine to treat diabetes in the present time. Based on the report of WHO, approximately 80% of the population use traditional medicine in developing countries (Efferth and Koch, 2011).

Ziziphus jujube (*Rhamnaceae* family) fruit (ZJF) is one of the medicinal plants that have been used since antiquity. This fruit is widely distributed in southern Asia and south-eastern Europe. This fruit contains high levels of calcium, phosphorus and vitamins A, C and B. Several constituents, which include flavonoids, procyanidin B2, saponins, tannins, some phenolic compounds (caffeic acid, catechin, epicatechin, chlorogenic acid and rutin) and polysaccharides, have been

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identified in ZJF (Choi *et al.*, 2011). Antioxidants and phytochemicals can improve glucose metabolism (suppression of glucose absorption via interaction with glucose transporters, and inhibition of α-glucosidase and decreased of hepatic glucose output) and blood lipids [inhibition of lipid peroxidation, low-density lipoprotein cholesterol (LDL-C) oxidation and the rate-limiting enzyme in cholesterol synthesis]. Because increased oxidative stress is considered as a leading cause of diabetic complications (Ha and Lee, 2005), the presence of these antioxidants in *jujube* fruit might have profitable effects in counterbalancing the heightened state of oxidative stress in patients with this disease (Li *et al.*, 2005).

In traditional medicine, *Ziziphus* is recommended for insomnia, liver and heart diseases, weakness, digestive disorders, convulsions, neurasthenia, anaemia, fever, diarrhoea, diabetes and high blood pressure (Bensky *et al.*, 1986). Several studies have shown the effects of this fruit on obesity and hyperlipidaemia (Sabzghabaee *et al.*, 2013), liver disorders (Shen *et al.*, 2009), hypertension (Koffi *et al.*, 2008) and infectious disease. Antidiabetic and hypolipidaemic effects of *Ziziphus* have been illustrated in streptozocin-induced diabetic rats (Glombitza *et al.*, 1994, Naftali *et al.*, 2009, Solati and Soleimani, 2010a, 2010b, Hemmati *et al.*, 2015). Avizeh *et al.* (2010) demonstrated that administration of *Ziziphus spina-christi* extract for 10 days is associated with reduction in blood glucose level and increase in serum insulin, in diabetic dogs. Improvement of lipid profile and body weight was observed with the consumption of ZJF powder in healthy volunteers after 3 months (Mostafa and Labban, 2013). Antioxidant effects of this fruit were also revealed in some animal studies (Shen *et al.*, 2009; Taati *et al.*, 2011).

Although some studies have reported the antidiabetic, antihyperlipidaemic and antioxidant effects of *Ziziphus* in animal models of diabetes, to the authors' knowledge there is no published article indicating the possible effects of ZJF infusion on blood glucose, serum lipid and antioxidant status in patients with T2DM. Therefore, this study determined the effects of brewed ZJF consumption (in the form of powder) on serum lipid profile, blood glucose and antioxidant activity in subjects with T2DM.

MATERIALS AND METHODS

Determining components of *Ziziphus jujube* fruit. Extract was prepared with classic or soaking method. Briefly, dried ripe fruits (peel and pulp) were poured into water and mixed by shaker for 24 h. After this time, the obtained solution was filtered by filter paper and dried by Rotary devices at 40°C. The fruit was homogenized with 2% metaphosphoric acid and then centrifuged at 10 000 rpm for 15 min. Eventually, it was passed through 0.45 μm membrane filter for determining vitamin C. Catechin, chlorogenic acid, vitamin C (with high-performance liquid chromatography), inulin, ferric-reducing antioxidant power and total phenolic, flavonoid content (with UV–visible spectrophotometer) were measured in this fruit. Total phenolic content was determined by Folin–Ciocalteu reagent (Velioglu *et al.*, 1998). A calibration curve of gallic acid (25 to 150 μg/mL in 80% methanol) was prepared. Aluminium chloride colourimetric method was used to measure the total flavonoid content in the plant (Zhishen *et al.*, 1999). Ferric-reducing antioxidant power assay was conducted according to the method of Benzie and Strain (Benzie and Strain, 1996). Five concentrations of $FeSO_4$ $7H_2O$ (12.5, 25, 50, 100 and 200 μmol/L) were used to draw a calibration curve. Colourimetric methods were used for determination of inulin (Schreiner, 1950). The used analytical column for identification of catechin and chlorogenic was Nucleodur 100–5 C18 (150 × 4.6 mm, 5 μm particle size, Waters, USA). The Eurospher-C18 column (300 × 4 mm, 5 μm, Knauer, Germany) was used to measure vitamin C.

Participants. The present clinical trial was conducted at Diabetes Research Centre, Yazd, Iran, from August 2014 to February 2015. The determination of sample size was carried out on the basis of the primitive information obtained from the study of Sabzghabaee *et al.* (2013) for fasting blood sugar (FBS) level. Considering $\alpha = 0.05$, effect size ($d = 0.55$) and power of equal to 80%, the size of sample calculated was 58 participants per group. Eligible participants were older than 30 years and had a confirmed diagnosis of diabetes type 2 according to American Diabetes Association for less than 10 years, with level of glycosylated haemoglobin (HbA1c) less than 9.5% and body mass index (BMI) $\geq$25 kg/m^2. Patients with a history of sensitivity to *Ziziphus* consumption (self-report), liver disease, renal failure, cancer and cardiovascular diseases (based on medical records); changing in routine treatment (including the dosage and type of the drugs) and physical activity; and starting insulin therapy were excluded from the current study. Participants who were consuming ZJF in the last 4 weeks or other medicinal herbs were not included. Those who consumed less than 70% of the packed sachet of the prescribed fruit were also excluded. Overall, 116 patients who expressed their willingness were included in the study (Fig. 1), and informed consent form was signed by all of them. Shahid Sadoughi University of Medical Sciences Ethics Committee approved this study, and it was registered in the Iranian Registry of Clinical Trials (www.irct.ir) under registry number (IRCT2014120510826N15).

Study design. This parallel-group randomized clinical trial was conducted in Diabetic Research Centre, Yazd, Iran. The participants were allocated into two groups (intervention or control) by using a computer-generated random sequence. In order to ensure blinding of this survey, the allocation of both groups was concealed from the assistants in the biochemical laboratories and statistician who analysed the data.

A balanced diet was prescribed for all of these 116 participants. The intervention group ($n = 58$) consumed 300 mL/day of ZJF infusion (10 g ZJF powder brewed in 100 mL boiling water for 10 min without filtering) three times/day before main meals (breakfast, lunch and dinner) for 12 weeks. The control group ($n = 58$) received only diet during this period. The participants were requested not to change their habitual physical activity. Adherence to the diets and consumption of ZJF

infusion were supported by the close relationship with patients via weekly phone interviews encouraging them to follow the study protocol. The patients were visited at baseline and weeks 2, 6, 10 and 12 of study for counting returned packed sachets, taking 24 h dietary recalls and physical activity records. Questionnaires were completed by a trained dietitian.

Diet. In both groups, a balanced diet was prescribed for each participant to provide about 500 kcal/day less than each subject's estimated daily energy requirement, based on resting energy expenditure and physical activity, calculated by the Harris–Benedict equation (Harris and Benedict, 1918). Macronutrient distribution (55% energy from carbohydrate, 15% energy from protein and 30% energy from fat) was the same in both groups. To help in selection of proper food, we provided a standard food exchange list for each patient concomitant with education. Energy and nutrient intakes were analysed using Nutritionist 4 software (First Databank, San Bruno, CA) modified for Iranian foods.

Dose, type of *Ziziphus* plant and intervention duration. First, the fruit was collected from a garden in Birjand City (Iran) in July 2014, and then the species was identified and authenticated by the laboratories of the School of Pharmacy and Pharmaceutical Sciences of Shahid Sadoughi University of Medical Sciences.

In native population of Northeast Iran, the infusion of ZJ is claimed as effective remedies for controlling and management of hyperlipidaemia and hyperglycaemia. Therefore, the participants were asked to use the brewed form of the ZJF powder. Dosage recommendation was selected in order not to exceed commonly the quantity of ZJ ingested as fruit, and a previous study described that the dose of 30 g/day of ZJ powder had therapeutic potential (Mostafa and Labban, 2013). After separating the kernels of ZJF from fruits, dried ripe fruits (peel and pulp) of ZJ were powdered by using

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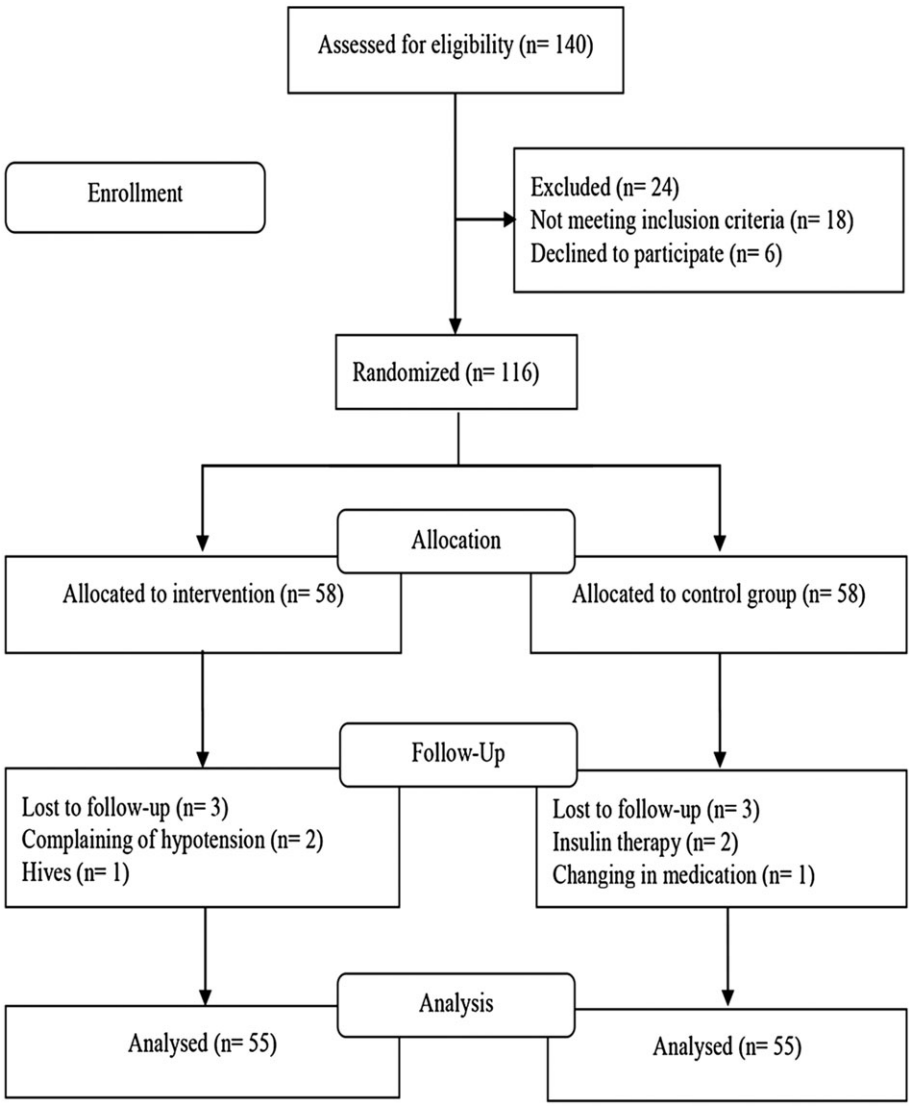

Figure 1. Summary of patient flow diagram.

mixer grinder, and eventually, the powder was packed in 10 g plastic sachets without any additional materials. Patients were educated to brew these fruits in a traditional way three separate times (before breakfast, lunch and dinner) throughout the day. The infusions were prepared by adding 100 mL of boiling water to 10 g ZJF powder, and after 10 min, the infusion was not filtered.

Measurements. A general questionnaire was completed through interview with each patient. All the measurements were carried out at baseline and after 12 weeks of the intervention.

Body weight of the subjects was measured while they were in light clothes and without shoes by using a digital scale (Seca, Hamburg, Germany) with 0.1 g accuracy. Height measurement was performed under standard protocols, using the non-stretched tape measure (Seca, Hamburg, Germany). For the calculation of BMI, the equation for BMI (weight in kg/height in metres squared) was used. Blood pressure was measured after the volunteers sat in a quiet room for 5 min.

For assessment of biochemical variables, a 10 mL sample of venous blood (after 12 h fasting) was taken from each participant. FBS and 2 h postprandial glucose (2hpp) were assessed by an enzymatic colourimetric method (glucose oxidize-peroxides), and HbA1c measurement was carried out using ion exchange chromatography. Colourimetric enzymatic assays were used to measure the level of the triglycerides (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) concentration. LDL-C and very LDL-C (VLDL-C) levels were computed according to the Friedewald equation (Friedewald *et al.*, 1972). If the serum triacylglycerol concentration exceeded 400 mg/dL, they were not calculated with this formula. All the biochemical assays were performed with the kits of Pars Azmoon, Iran. 2,2′-Diphenyl-1-picrylhydracyl was used to evaluate total antioxidant capacity (TAC) based upon Janaszewska method (Janaszewska and Bartosz, 2002). Serum malondialdehyde (MDA) concentration was estimated using thiobarbituric acid reactive substances assay at 532 (Del Rio *et al.*, 2003).

STATISTICAL ANALYSIS

Normally distributed continuous and non-normally distributed data (determined by Kolmogorov–Smirnov and Shapiro–Wilk tests) are presented as mean ± SD. Independent sample *t*-test was used for comparison between groups and paired sample *t*-test for comparison within group differences. Logarithmic transformation was used to normalize distribution of the skewed variables, and significance was evaluated by parametric analysis. After computing the changes from baseline by subtracting the baseline values from final values, analysis of covariance was used to evaluate differences between groups, adjusting for age, gender, duration of diabetes, baseline values, intake of hypoglycaemic, hypolipidaemic and hypotensive drugs as the covariates. Results with *p*-values less than 0.05 were regarded as statistically significant. All the statistical analyses were performed with SPSS version 16 (SPSS Inc., Chicago, IL, USA).

RESULTS

The constituents of the ZJF are summarized in Table 1. A total of 110 out of the 116 participants with mean body weight and BMI of 78.76 ± 13 and 29.89 ± 5.03 completed this clinical trial. The numbers of menopausal women were eight and five in intervention and control groups, respectively ($p = 0.55$). Participants compliance to the infusion consumption was more than 96% based on the remaining ZJF powder in the packed sachets, and also, consumption of ZJF infusion (300 mL/day) was well tolerated by the patients during the intervention. Baseline characteristics and anthropometric parameters of subjects are shown in Table 2. Distribution of participants in terms of age, gender, duration of diabetes and taking oral antidiabetic, antihyperlipidaemic and antihypertensive medications were not significantly different between the two groups.

Based on 24 h dietary recall, no statistically significant difference was found between the two groups in dietary intakes of energy, carbohydrate, protein and fat at baseline and after 12 weeks of study, while significant within-group reductions in all these variables were observed for the ZJF and control groups (Table 2). These findings demonstrated a perfect observance of the prescribed diet. Physical activity was assessed with three random days (non-consecutive) records throughout the study. No significant difference was observed in the physical activity levels among the groups during the intervention.

Table 1. Chemical composition of ZJF used in the study

Constituents	Identification method	λ max(nm)	Amount of acquired compound
Vitamin C (mg/100 g DW)	HPLC	210	81.3
Catechin (mg/100 g DW)	HPLC	280	17.97
Chlorogenic acid (mg/100 g DW)	HPLC	375	0.466
Inulin (mg/g DW)	UV–visible spectrophotometer	485	327.45
Total phenolic (mg GAE/g DW)	UV–visible spectrophotometer	725	8.63 ± 1.21
Total flavonoid (mg CE/g DW)	UV–visible spectrophotometer	510	11.3 ± 0.95
FRAP assay (mmol Fe^{2+}/g DW)	UV–visible spectrophotometer	585	156.1 ± 2.36

mg GAE/g DW, milligrams of Gallic acid equivalents per gram of dry weight; mg CE/g DW, mg catechin equivalent per gram dry weight; FRAP, ferric-reducing antioxidant power; ZJF, *Ziziphus jujube* fruit.

Table 2. General characteristics and dietary intakes of the study participants

	Diet only (n = 55)		Diet + *Ziziphus* fruit (n = 55)		p-value*		
Age (years)	50.69 ± 1.08		48.98 ± 1.06		0.26		
Female/male (n)	35/20		34/21		0.84		
Height (cm)	161.51 ± 1.01		161.36 ± 0.9		0.90		
Duration of diabetes (years)	3.43 ± 0.29		3.68 ± 0.34		0.57		
Smokers (%)	18.2		23.6		0.48		
SBP (mm Hg)	130.0 ± 15.01		130.06 ± 14.65		0.84		
DBP (mm Hg)	82.36 ± 8.26		83.63 ± 7.96		0.41		
Antidiabetic medication, n (%)							
Gliclazide	5 (9.1)		4 (7.3)		0.98		
Metformin	55 (100)		55 (100)				
Glibenclamide	21 (38.2)		17 (30.9)		0.54		
Antilipidaemic medication, n (%)							
Atorvastatin	26 (47.3)		31 (56.4)		0.44		
Lovastatin	5 (9.1)		7 (12.7)		0.76		
Gemfibrozil	7 (12.7)		6 (10.9)		0.98		
Antihypertensive medication, n (%)							
Losartan	25 (51)		24 (49)		0.98		
Amlodipine	7 (12.7)		5 (9.1)		0.76		
	Baseline	WK12	Baseline	WK12	Baseline	WK12	Group difference
Energy (kcal/day)	1941.14 ± 287.90	1718.74 ± 179.72**	1976.32 ± 282.82	1694.09 ± 140.52**	0.32	0.53	0.1
Carbohydrate (g/day,	278.32 ± 51.90	237.45 ± 27.35**	280.06 ± 50.40	232.86 ± 24.32**	0.80	0.35	0.45
% of energy)	56.30	54.7	55.3	54.45			
Protein (g/day,	67.58 ± 14.22	64.02 ± 10.55**	71.18 ± 12.60	63.25 ± 8.14**		0.67	0.11
% of energy)	13.83	14.7	14.09	14.78	0.16		
Fat (g/day,	64.30 ± 13.85	59.23 ± 12.30**	68.28 ± 14.01	58.61 ± 6.37**		0.71	0.08
% of energy)	29.70	30.60	30.43	30.76	0.13		

Data are expressed as mean ± SD or number (percentage) of patients. SBP, systolic blood pressure; DBP, diastolic blood pressure.
*Obtained from independent samples t-test for continuous variables and Chi-square for categorical ones.
**$p < 0.05$, paired samples t-test.

Glycaemic indices and lipid profile

The mean values of the glycaemic indices and serum lipid profiles at baseline and end of the intervention are illustrated in Table 3. Independent sample t-test showed that the baseline levels of FBS, 2hpp, HbA1c and serum lipids were not different between two groups. Compared with the control group, the value of HbA1c, TC, LDL-C, TC/HDL-C and LDL-C/HDL-C ratios significantly decreased at the end of the study (all $p < 0.05$), whereas after 12 weeks of ZJF consumption, FBS ($p = 0.08$), HDL-C ($p = 0.81$), TG and VLDL-C ($p = 0.06$) were not significantly different between two groups. Although concentration of 2hpp glucose was different between the two groups at the end of the trial ($p > 0.01$), there was no marked difference between mean changes of this variable in both groups in crude and adjusted models ($p > 0.05$). As shown in Table 3, no significant effects of ZJF on FBS and HDL-C were found in either the crude or adjusted models. A comparison of changes in plasma HbA1c, TC, LDL-C, TG, VLDL-C, LDL-C/HDL-C and TC/HDL-C ratios between groups indicated significant reduction in the crude model ($p < 0.05$), and these differences remained significant after the influence of confounding factors was taken into account.

Antioxidant status

The serum levels of TAC and MDA did not differ significantly between the two groups at baseline ($p = 0.14$, $p = 0.30$) and end of this trial ($p = 0.17$, $p = 0.97$), respectively. A comparison of changes in TAC between the two groups revealed significant difference in the crude model ($p = 0.03$); however, after adjustment for potential confounding variables, there were no significant differences in changes in TAC between the two groups ($p = 0.13$). Serum MDA level was decreased in both groups, but the differences in the mean changes in MDA were not significant, and the present results with adjustment for confounders did not change (Table 3).

DISCUSSION

This randomized trial showed that ZJF at a dose of 30 g/day for 12 weeks had beneficial effects on HbA1c, TC, LDL-C, TG, VLDL-C, LDL-C/HDL-C and TC/HDL-C ratios compared with the control group. In addition, the level of HDL-C and TAC increased and MDA reduced compared with their baseline values in the ZJF group. Although the effects of medication treatment on glycaemic control and hyperlipidaemia cannot

Table 3. Glycaemic status, lipid profiles and antioxidant status in diabetic patients at baseline and after 12 weeks of intervention

	Diet only (n = 55)			Diet + *Ziziphus* fruit (n = 55)			p-value[a]			
									Group difference[d]	
	Baseline	WK12	Change[c]	Baseline	WK12	change	Baseline	WK12	Crude	Model 1
FBS (mg/dL)	150.78 ± 27.63	136.6 ± 23.05[bc]	−14.18 ± 18.32	146.12 ± 24.32	129.24 ± 21.83[c]	−16.89 ± 18.89	0.35	0.08	0.44	0.18
2hpp glucose (mg/dL)[†a]	214.22 ± 63.65	181.36 ± 37.2[b]	−32.85 ± 46.40	196.43 ± 50.19	161.61 ± 42.77[b]	−34.81 ± 44.14	0.14	0.01	0.82	0.07
HbA1c (%)	8.08 ± 0.84	7.64 ± 0.71[b]	−0.44 ± 0.55	7.96 ± 0.71	7.28 ± 0.59[b]	−0.68 ± 0.65	0.42	0.005	0.03	0.002
TC (mg/dL)[†]	174.31 ± 35.57	163.09 ± 34.86[c]	−11.21 ± 29.98	172.89 ± 25.21	148.6 ± 26.64[b]	−24.29 ± 28.89	0.96	0.02	0.02	0.005
LDL-C (mg/dL)[†]	93.91 ± 27.62	87.36 ± 29.47	−6.55 ± 27.82	92.66 ± 26.07	72.81 ± 21.18[b]	−19.85 ± 27.62	0.82	0.004	0.01	0.002
HDL-C (mg/dL)[†]	45.05 ± 7.09	46.34 ± 5.87	1.29 ± 7.05	44.85 ± 6.89	46.38 ± 4.2[b]	1.52 ± 6.75	0.89	0.81	0.85	0.88
TG (mg/dL)[†]	180.33 ± 61.92	153.16 ± 44.4[b]	−27.16 ± 46.84	181.67 ± 67.68	138.36 ± 53.28[b]	−43.3 ± 39.26	0.95	0.06	0.05	0.01
LDL-C/HDL-C ratio	2.1 ± 0.59	1.89 ± 0.61[b]	−0.2 ± 0.72	2.13 ± 0.77	1.56 ± 0.42[b]	−0.56 ± 0.80	0.88	0.003	0.01	0.001
TC/HDL-C ratio[†]	3.89 ± 0.65	3.53 ± 0.68[b]	−0.35 ± 0.77	3.93 ± 0.81	3.2 ± 0.49[b]	−0.73 ± 0.94	0.87	0.005	0.02	0.002
VLDL (mg/dL)[†]	36.06 ± 12.38	30.63 ± 8.88[b]	−5.43 ± 9.36	36.33 ± 13.53	27.67 ± 10.65[b]	−8.66 ± 7.85	0.95	0.06	0.05	0.01
TAC (%)	49.44 ± 22.90	52.79 ± 20.86	3.35 ± 33.24	43.68 ± 18.00	58.34 ± 18.87[c]	14.66 ± 19.53	0.14	0.17	0.03	0.13
MDA (μmol/L)	1.46 ± 0.74	1.19 ± 0.44[c]	−0.27 ± 0.78	1.59 ± 0.53	1.18 ± 0.58[c]	−0.40 ± 0.73	0.30	0.97	0.37	0.77

Data are shown as mean ± SD.

[†]p-value was obtained after variables were log transformed to normalize the distributions.

[a]Resulted from independent samples t-test.

[b]$p < 0.05$, paired samples t-test.

[c]Change from baseline.

[d]Unadjusted model compares the raw changes over 12 weeks between control and *Ziziphus* fruit groups; model 1 adjusted for age, gender, duration of diabetes, baseline values and intake of hypoglycaemic, hypolipidaemic and hypotensive drugs as the covariates.

FBS, fasting blood sugar; 2hpp, 2 h postprandial glucose; HbA1c, glycosylated haemoglobin; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; LDL-C/HDL-C ratio, low-density lipoprotein cholesterol to high-density lipoprotein cholesterol ratio; TC/HDL-C ratio, total cholesterol to high-density lipoprotein cholesterol ratio; VLDL-C, very low-density lipoprotein cholesterol; TAC, total antioxidant capacity; MDA, malondialdehyde.

be ignored, these two groups were similar in terms of type and dosage of used drugs. Moreover, the results were adjusted for medications used by patients. Therefore, the positive effects we found would be independent of taking oral agents.

There are some experimental studies on the effect of *Ziziphus* on glycaemic and antioxidant status and lipid profiles (Glombitza *et al.*, 1994; Naftali *et al.*, 2009). Based on this literature review, this is the first study that has assessed the effects of the fruit on glycaemic control (FBS, 2hpp and HbA1c), serum lipid profiles (TC, LDL-C, TG, HDL-C, VLDL-C, LDL-C/HDL-C and TC/HDL-C ratios) and antioxidant status (TAC and MDA) in patients with T2DM who were on oral antidiabetic drugs.

Sabzghabaee *et al.* (2013) showed significant improvement in TC, LDL-C and FBS in obese adolescents with dyslipidaemia who used 15 g/day of ZJF for 1 month. Consumption of ZJ powder in healthy individuals for 3 months resulted in decrease in TC, LDL-C and TG and a slight increase in HDL-C (Mostafa and Labban, 2013). Similar results have also been reported in rats that received hepatotoxic agents (Yossef *et al.*, 2011). In a study on diabetic rats that were gavaged with extract of ZJF, TG levels decreased but the levels of LDL-C and HDL-C were not significantly changed after 14 days (Solati and Soleimani, 2010a, 2010b). Water extract of ZJF indicated no significant change in HDL-C level in diabetic rats (Solati and Soleimani, 2010a, 2010b).

No significant effects of ZJF on serum FBS and 2hpp levels were found. In contrast to the present findings, Avizeh *et al.* (2010) and Hussein *et al.* (2006) observed favourably the influence of ZF on blood glucose level. The same finding has also been reported in adolescents with dyslipidaemia (Sabzghabaee *et al.*, 2013). The consumption of ZJF extracts (25 and 100 mg/kg) for 2 weeks improved blood glucose in diabetic rats besides increased adiponectin levels (Hemmati *et al.*, 2015). The discrepancies in the findings may be related to the different forms of ZJF, difference in the types of studied populations and the species and geographical growth location. The results of the present study with regard to the HbA1c are in line with the study, indicating the effect of ZJ consumption on HbA1c in diabetic rats (Goli-malekabadi *et al.*, 2014). According to the results of this study and previous studies, this fruit is potentially a rich source of ascorbic acid. Vitamin C can play an important role in the suppression of free radicals. On the other hand, the structure of ascorbic acid is similar to glucose; therefore, it is effective in prevention of non-enzymatic protein glycosylation (Ceriello *et al.*, 1992). Thus, the effect of ZJF on HbA1c can be related to this vitamin.

Consumption of ZJ has led to a non-significant enhancement in serum TAC. Although, according to paired *t*-test between baseline and follow-up, serum levels of MDA significantly decreased in both groups ($p < 0.05$), no significant differences were observed in MDA between the ZJF and control groups. This finding is in agreement with the results of Taati *et al.* (2011) on rat after administration of ZJ extract. However, some studies found beneficial effect of ZJF consumption on TAC and MDA levels (Shen *et al.*, 2009; Yossef *et al.*, 2011; Goli-malekabadi *et al.*, 2014). As regards most of the articles published that has not considered the effect of some confounding variables, it can provide a reason for discrepant findings. Several mechanisms can show the beneficial effects of ZJF on lipid profiles, and glycaemic and antioxidant biomarkers. *Ziziphus* contains high amounts of pectin, inulin and unsaturated fatty acids, so the hypolipidaemic effect of ZJF might be due to their presence (Lunn and Theobald, 2006; Pourghassem Gargari *et al.*, 2013; Sabzghabaee *et al.*, 2013). In this study, the analysis of ZJF showed the high concentration of inulin. The high saponins content of ZJF may also describe its positive impact on plasma lipids (Goyal and Grewal, 2003; Choi *et al.*, 2011). The presence of phytosterols in ZJF inhibits the intestinal cholesterol absorption, thus helping to reduce TC and LDL-C, whereas most studies reported that they had no effect on HDL-C (Miettinen *et al.*, 1995). ZJF may have important role on lipid and glucose metabolism via activation of adiponectin signalling pathways (Qiao *et al.*, 2008; Hemmati *et al.*, 2015). The main phenolic compounds in ZJF are ferulic acid, catechin and rutin, which could in turn affect glucose metabolism. They can depict the hypoglycaemic properties by inhibiting intestinal α-glycosidase activity, decreasing hepatic glucose production and dealing with glucose transporters. On the other hand, it has been suggested that ZJF might play a protective role against acute/chronic inflammatory reactions, probably by attenuating NOS activity (Ohnishi *et al.*, 2004; Nagao *et al.*, 2009; San and Yildirim, 2010). Chlorogenic acid in this fruit may slow down carbohydrate absorption by inhibiting intestinal glucose transport (McCarty, 2005). We also showed that the content of catechin and chlorogenic acid in ZJF was considerable. The effect of ZJF on oxidative stress can be related to its high natural antioxidant components such as flavonoids, polysaccharides fractions and vitamin C, which can play a role in inhibiting oxidative stress caused in T2DM by attenuating peroxidation of membrane lipids and decelerating other harmful effects of free radicals (Li *et al.*, 2005; Choi *et al.*, 2011).

Strengths of this study include the relatively large sample size, randomized design and taking confounders including baselines levels of biomarkers and duration of diabetes into account. Another strength point is that similar diet was prescribed for both groups; thus, dietary factors could not confound the results. Nevertheless, this trial had some limitations. The results cannot be generalized to other T2DM (those who are receiving insulin injection) because it was conducted among diabetic patients who were treated with oral hypoglyceamic agent. It was better to control physical activity by a valid questionnaire such as the international physical activity questionnaire and consider placebo group. The differences in some results might be somewhat due to discrepancies in the type and dose of the prescribed ZJF and the types of studied populations. Therefore, designing double-blind placebo-controlled studies with longer duration and different dosages of ZJF is recommended to determine the appropriate doses in diabetic patients.

CONCLUSION

Consumption of ZJF in patients with T2DM had beneficial effects on HbA1c, lipid profiles and, partially, antioxidant status. Further studies are required to

conclusively prove the efficacy of ZJF in the prevention and treatment of T2DM and to elucidate the metabolic basis of its hypoglycaemic and hypolipidaemic activity.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

REFERENCES

Avizeh R, Najafzadeh H, Pourmahdi M, Mirzaee M. 2010. Effect of glibenclamide and fruit extract of *Zizyphus spina-christi* on alloxan-induced diabetic dogs. *J Appl Res Vet Med* **8**(2): 109–113.

Bensky D, Gamble A, Kaptchuk TJ. 1986. Chinese herbal medicine: materia medica. *Eastland Press* 580–581.

Benzie IF, Strain J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Anal Biochem* **239**(1): 70–76.

Ceriello A, Quatraro A, Giugliano D. 1992. New insights on non-enzymatic glycosylation may lead to therapeutic approaches for the prevention of diabetic complications. *Diabet Med* **9**(3): 297–299.

Choi S-H, Ahn J-B, Kozukue N, Levin CE, Friedman M. 2011. Distribution of free amino acids, flavonoids, total phenolics, and antioxidative activities of *jujube (Ziziphus jujuba)* fruits and seeds harvested from plants grown in Korea. *J Agric Food Chem* **59**(12): 6594–6604.

Del Rio D, Pellegrini N, Colombi B, *et al.* 2003. Rapid fluorimetric method to detect total plasma malondialdehyde with mild derivatization conditions. *Clin Chem* **49**(4): 690–692.

Efferth T, Koch E. 2011. Complex interactions between phytochemicals. The multi-target therapeutic concept of phytotherapy. *Curr Drug Targets* **12**(1): 122–132.

Friedewald WT, Levy RI, Fredrickson DS. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* **18**(6): 499–502.

Glombitza K, Mahran G, Mirhom Y, Michel K, Motawi T. 1994. Hypoglycemic and antihyperglycemic effects of *Zizyphus spina-christi* in rats. *Planta Med* **60**(3): 244–247.

Goli-malekabadi N, Asgary S, Rashidi B, *et al.* 2014. The protective effects of *Ziziphus vulgaris L*. fruits on biochemical and histological abnormalities induced by diabetes in rats. *J Compl Int Med* **11**(3): 171–177.

Goyal R, Grewal RB. 2003. The influence of teent (*Capparis decidua*) on human plasma triglycerides, total lipids and phospholipids. *Nutr Health* **17**(1): 71–76.

Ha H, Lee HB. 2005. Reactive oxygen species amplify glucose signalling in renal cells cultured under high glucose and in diabetic kidney. *Nephrology* **10**(s2): S7–S10.

Harris JA, Benedict FG. 1918. A biometric study of human basal metabolism. *Proc Natl Acad Sci U S A* **4**(12): 370.

Hemmati M, Asghari S, Zohoori E, Karamian M. 2015. Hypoglycemic effects of three Iranian edible plants; *jujube*, barberry and saffron: correlation with serum adiponectin level. *Pak J Pharm Sci* **28**(6): 2095–2099.

Hussein HM, El-Sayed EM, Said AA. 2006. Antihyperglycemic, antihyperlipidemic and antioxidant effects of *Zizyphus spina christi* and *Zizyphus jujuba* in alloxan diabetic rats. *Int J Pharm* **2**: 563–570.

Janaszewska A, Bartosz G. 2002. Assay of total antioxidant capacity: comparison of four methods as applied to human blood plasma. *Scand J Clin Lab Invest* **62**(3): 231–236.

Koffi A, Traore F, Adjoungoua A, Diafouka F. 2008. Effets pharmacologiques de *Ziziphus mauritiana Lam.(Rhamnacées)* sur la pression artérielle de lapin. *Phytothérapie* **6**(4): 219–227.

Li J-w, Ding S-d, Ding X-l. 2005. Comparison of antioxidant capacities of extracts from five cultivars of Chinese jujube. *Process Biochem* **40**(11): 3607–3613.

Lunn J, Theobald H. 2006. The health effects of dietary unsaturated fatty acids. *Nutr Bull* **31**(3): 178–224.

McCarty MF. 2005. Nutraceutical resources for diabetes prevention – an update. *Med Hypotheses* **64**(1): 151–158.

Miettinen TA, Puska P, Gylling H, Vanhanen H, Vartiainen E. 1995. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. *N Engl J Med* **333**(20): 1308–1312.

Mostafa UE-S, Labban L. 2013. Effect of *Zizyphus jujuba* on serum lipid profile and some anthropometric measurements. *Adv Med Plant Res* **1**(3): 49–55.

Naftali T, Feingelernt H, Lesin Y, Rauchwarger A, Konikoff FM. 2009. *Ziziphus jujuba* extract for the treatment of chronic idiopathic constipation: a controlled clinical trial. *Digestion* **78**(4): 224–228.

Nagao T, Meguro S, Hase T, *et al.* 2009. A catechin-rich beverage improves obesity and blood glucose control in patients with type 2 diabetes. *Obesity* **17**(2): 310–317.

Nathan DM. 1993. Long-term complications of diabetes mellitus. *N Engl J Med* **328**(23): 1676–1685.

Ohnishi M, Matuo T, Tsuno T, *et al.* 2004. Antioxidant activity and hypoglycemic effect of ferulic acid in STZ-induced diabetic mice and KK-Ay mice. *Biofactors* **21**(1–4): 315–319.

Pourghassem Gargari B, Dehghan P, Mirtaheri E, Aliasgarzadeh A. 2013. Effects of inulin on lipid profile, inflammation and blood pressure in women with type 2 diabetes: a randomized controlled trial. *J Ardabil University Med Sci* **13**(4): 359–370.

Qiao L, Zou C, van der Westhuyzen DR, Shao J. 2008. Adiponectin reduces plasma triglyceride by increasing VLDL triglyceride catabolism. *Diabetes* **57**(7): 1824–1833.

Sabzghabaee AM, Khayam I, Kelishadi R, *et al.* 2013. Effect of *Zizyphus jujuba* fruits on dyslipidemia in obese adolescents: a triple-masked randomized controlled clinical trial. *Mediev Archaeol* **67**(3): 156–159.

San B, Yildirim AN. 2010. Phenolic, alpha-tocopherol, beta-carotene and fatty acid composition of four promising *jujube (Ziziphus jujuba Miller)* selections. *J Food Compos Anal* **23**(7): 706–710.

Schreiner GE. 1950. Determination of inulin by means of resorcinol. *Exp Biol Med* **74**(1): 117–120.

Shen X, Tang Y, Yang R, Yu L, Fang T, Duan J-a. 2009. The protective effect of *Zizyphus jujube* fruit on carbon tetrachloride-induced hepatic injury in mice by anti-oxidative activities. *J Ethnopharmacol* **122**(3): 555–560.

Solati J, Soleimani N. 2010a. Antidiabetic effects of ethanolic extract of *Ziziphus vulgaris L*. in streptozocin induced. *Physiol Pharmacol* **14**(2): 174–180.

Solati J, Soleimani N. 2010b. Antihyperglycemic and antihyperlipidemic effects of *Ziziphus vulgaris L*. onreptozocin-induced diabetic adult male Wistar rats. *Acta Diabetol* **47**(1): 219–223.

Taati M, Alirezaei M, Meshkatalsadat M, Rasoulian B, Kheradmand A, Neamati S. 2011. Antioxidant effects of aqueous fruit extract of *Ziziphus jujuba* on ethanol-induced oxidative stress in the rat testes. *Iranian J Vet Res* **12**(1): 39–45.

Velioglu Y, Mazza G, Gao L, Oomah B. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J Agric Food Chem* **46**(10): 4113–4117.

Yossef H, Khedr AA, Mahran MZ. 2011. Hepatoprotective activity and antioxidant effects of El Nabka (*Zizyphus spina-christi*) fruits on rats hepatotoxicity induced by carbon tetrachloride. *Nat Sci* **9**(2): 1–7.

Zhao Y, Yang X, Ren D, Wang D, Xuan Y. 2014. Preventive effects of *jujube* polysaccharides on fructose-induced insulin resistance and dyslipidemia in mice. *Food Funct* **5**(8): 1771–1778.

Zhishen J, Mengcheng T, Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* **64**(4): 555–559.

