Original Article

Effect of dried Ziziphus Jujube Consumption on Plasma Insulin, Blood Pressure, Oxidative Stress, and Advanced Glycation End Products in Diabetic Patients with Excess Weight: A Randomized Controlled Trial

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Abstract

Background: Poor glycemic control and oxidative stress can accelerate the development of type 2 diabetes (T2D) complications. Recently, the potential benefits of herbs in managing T2D have received more attention. Therefore, we examined the effect of consuming dried Ziziphus jujube (ZJ) on plasma insulin, blood pressure, oxidative stress markers, and advanced glycation end products (AGEs) in T2D patients with excess weight. **Methods:** Forty-eight diabetic patients with excess weight were selected and randomly assigned to the ZJ group (n = 24) or the control group (n = 24). The case group received 30g of dried ZJ fruit daily for three months. Concentrations of fasting insulin, malondialdehyde (MDA), total antioxidant capacity (TAC), carboxymethyl lysine (CML), and blood pressure (BP) were measured. **Results:** After adjusting for baseline values, the ZJ group showed a significant decrease in systolic BP (up to 7 mmHg) and diastolic BP (up to 5 mmHg) compared to the control group ($P < 0.05$). Moreover, plasma insulin levels significantly decreased (up to 4.6 mIU/L) in the ZJ group compared to the control group ($P < 0.05$). However, there were no significant differences in the mean changes of plasma TAC, MDA, and CML between the two groups. **Conclusions:** The present study suggests that consuming 30g/day of dried ZJ fruit may improve some cardiometabolic profiles, including plasma insulin and blood pressure in T2D patients with excess weight. **Trial registration:** The present clinical trial has been enrolled in the Iranian registry of clinical trials with the registration number IRCT20181210041913N1.

Keywords: *Advanced glycation end products, blood pressure, oxidative stress, plasma insulin, type 2 diabetes, ziziphus jujube*

Introduction

Type 2 diabetes (T2D) is a major multifactorial public health issue and a leading risk factor for cardiovascular diseases (CVDs) and mortality.[1,2] The prevalence of T2D is rising globally due to increased exposure to unhealthy lifestyle determinants including unhealthy dietary patterns, sedentary behavior, and adiposity.[3,4] Without appropriate management, T2D can lead to serious microvascular and macrovascular complications, including nephropathy, retinopathy, CVDs, and peripheral neuropathies.[5] Various metabolic disorders, such as dysglycemia, hyperinsulinemia, elevated weight, high blood pressure, and oxidative stress, are mainly responsible for these complications in patients with T2D.[6]

Along with using anti-glycemic medications, dietary pattern modification, weight management, and increased physical activity are primary strategies for controlling T2D.[7,8] However, life-threatening complications can still be prevalent in T2D patients, especially those who are obese or overweight. In such cases, poor glycemic control combined with high oxidative stress makes patients more susceptible to various complications of diabetes. Therefore, in addition to standard medical nutrition therapy, recent investigations have focused on using complementary herbal medicine such as Ziziphus jujube (ZJ), barberry, saffron, and turmeric to treat or control diabetes complications due to their high content of phytochemicals and antioxidants.[9-12]

Ziziphus jujube, a common herbal product from the Rhamnaceae family, is rich in antioxidant vitamins, minerals, and bioactive compounds, including

How to cite this article: Farhadnejad H, Hedayati M, Sahranavard S, Jahromi MK, Asghari G, Teymoori F, *et al.* Effect of dried Ziziphus jujube consumption on plasma insulin, blood pressure, oxidative stress, and advanced glycation end products in diabetic patients with excess weight: A randomized controlled trial. Int J Prev Med 2025;16:49.

Access this article online

Website: www.ijpvmjournal.net/www.ijpm.ir

DOI: 10.4103/ijpvm.ijpvm_144_24

Quick Response Code:

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flavonoids, polysaccharides, triterpenic acids, saponins, and some phenolic compounds. These components are suggested to play important roles in health promotion and the management of chronic diseases through various physiological functions such as anticancer effects, antioxidant, immune system regulation, nervous system protection, sedative, and antiviral effects.[13,14] In addition, experimental studies have reported the anti-hyperglycemic, anti-hypertensive, anti-hyperlipidemic, and antioxidant effects of ZJ in diabetic rats.[15-17]

Previous studies on the effects of ZJ intake on clinical markers in T2D patients have yielded controversial results.[18,19] For example, the study by Yazdanpanah *et al*.[18] found that ZJ intake did not have beneficial effects on blood glucose and oxidative stress indices. Another study reported that ZJ consumption reduced fasting plasma glucose and serum insulin in T2D patients; however, no beneficial effects of ZJ on blood pressure were observed.[19] Therefore, there is still insufficient evidence from human studies to conclusively demonstrate the significant effect of ZJ on cardio-metabolic parameters. Moreover, no studies have examined the effect of ZJ on advanced glycation end products (AGEs) in T2D patients. Given that overweight and obese T2D patients may be more susceptible to T2D complications due to poor glycemic control and oxidative stress,[20-22] we designed a randomized controlled clinical trial to investigate the effects of dried ZJ intake on plasma insulin, blood pressure, oxidative stress markers, and AGEs in T2D patients with excess weight, independent of its impact on weight loss.

Materials and Methods

Study design

The current study follows the 12-week, parallel-assigned, randomized controlled clinical trial (RCT) protocol detailed in a previous publication.[12] This trial involved 48 participants, aged 30 to 65 years, diagnosed with T2D, and having a body mass index (BMI) of 25 kg/m² or more. Using convenience sampling, participants were recruited from the Endocrinology Clinic in the Hospital of Ayatollah Taleghani in Tehran, Iran. Other inclusion criteria for the present trial were a history of diabetes for 1 to 10 years, systolic blood pressure (SBP) above 120 mmHg, diastolic blood pressure (DBP) above 80 mmHg, and not using insulin. Also, individuals were not included in this study if they used oral hypoglycemic drugs other than metformin or had liver failure, chronic inflammatory diseases, hypothyroidism, cardiovascular disease, kidney failure, or a history of cancer. Participants were also excluded if they reported allergies or complaints about ZJ consumption, consumed alcohol, regularly used dietary supplements, had opioid addiction, or used anti-inflammatory drugs. Additionally, pregnant or lactating patients and those who underwent significant medication changes prescribed by an endocrinologist were excluded.

During the first visit of our study, we explained the study's objective and protocol to the participants and obtained their informed consent. In the subsequent visit (baseline phase of the study), the participants were randomly assigned to either the intervention group or the control group using the block randomization method.[12] We used block sizes of four and created six possible balanced combinations of two control (C) and two ZJ (Z) participants (CCZZ, CZZC, ZZCC, CZCZ, ZCZC, and ZCCZ) (6 blocks). We then randomly selected the blocks using convenience sampling to ensure that all participants were assigned to either the ZJ or control group. An independent statistician performed the block randomization, with the participants and investigators (assistant laboratory measurements and statistical analysis) concealed from the randomization process until the statistical analysis was completed.

Participants in the ZJ group were administered 30 g/day of dried ZJ fruit and given healthy dietary recommendations based on the food guide pyramid for 12 weeks. The control group received only healthy dietary recommendations according to the food guide pyramid. We supplied an adequate amount of dried ZJ fruit for six weeks at both the baseline and at the end of the sixth week for the intervention group. The intervention group consumed 30 grams per day of dried ZJ fruit as 10 grams three times/day before main meals, including breakfast, lunch, and dinner for 12 weeks. The nutritional and bioactive components of the dried ZJ fruit used in our study were previously analyzed in detail.[12] A standard method for nutritional analysis showed that the dried ZJ contained 0.46 mg of chlorogenic acid, 81.3 mg of vitamin C, and 17.97 mg of catechin per 100 g of dried weight. In addition, this fruit contained 327.45 mg of inulin per 1 g of dried weight of jujube, 8.63 mg of gallic acid equivalents per 1 gram of dried weight of total phenolic, 11.3 mg of catechin equivalent per 1 g dried weight of total flavonoid, and 156.1 mmol Fe2 + per 1 g of dry weight (based on FRAP assay).[18] We asked all participants not to make any changes to their food habits, physical activity, or medications during the 12-week follow-up period of the study

To ensure compliance with the intake of 30 grams/day of dried ZJ fruit, participants in the intervention group were

provided with pre-packaged ZJ fruit packs, each containing 30 grams, for a six-week period. Clear instructions were given to return any unused packs at the sixth and twelfth follow-up meetings. Participants were also instructed to place the used ZJ kernels inside each pack and bring them to the follow-up visits at the 6th and 12th weeks of the study. Additionally, we contacted participants by phone twice a week to monitor their cooperation and adherence to the study protocol. At the beginning and end of the 12-week follow-up, we collected a fasting 10 cc blood sample from each participant using a standard protocol. The blood samples were centrifuged at 2000 rpm within 30-45 minutes of collection and separated into small aliquots before being frozen at -70°C until biochemical variables measurements could be conducted. The Endocrine Research Institute laboratory conducted the blood sample analysis.

Measurements

As detailed in a previous study,[12] a standard questionnaire was used to obtain participants' general information on demographics, disease history, drug usage, and smoking status at baseline. To ensure participants' dietary intake remained consistent throughout the 12-week follow-up period, we collected dietary intake data using three 24-hour dietary recalls (covering two regular workdays and one weekend day) through face-to-face and telephone interviews at the beginning and end of the study. We used Nutritionist 4 software (N Squared Computing, San Bruno, CA, USA) to analyze participants' nutritional data, including their energy and nutrient intake.

We measured anthropometric-related variables, including weight and height, at the beginning and end of the 12-week follow-up period using accurate measurement tools and standard methods, as described in a previous study.[12] BMI was calculated as the ratio of weight (in kg) to height (in m2) squared. We measured blood pressure (systolic and diastolic) twice on the right arm using a standardized mercury sphygmomanometer with an accuracy of 2 mmHg, and the mean of the two measurements was recorded as the patient's blood pressure. Physical activity (PA) levels were assessed using a short form of the International Physical Activity Questionnaire (IPAQ)[23] and expressed as metabolic equivalent minutes per week (MET-minutes/week). Participants were categorized into three groups based on their PA levels: Low active (below 600 MET-minutes/week), moderately active (600 to 2,999 MET-minutes/week), and highly active (at least 3,000 MET-minutes/week).

We used an auto-analyzer with 0.75 micU/mL sensitivity and an insulin ELISA kit (Monobind Inc) to determine insulin plasma levels, with an intra-assay coefficient of variation (CV) of 1.15%. We calculated the homeostasis model assessment of insulin resistance (HOMA-IR) as fasting insulin (in microU/L) multiplied by fasting glucose (in nmol/L) divided by 22.5.[24] We determined plasma malondialdehyde (MDA) levels using a colorimetric method and commercial kits (ZellBio GmbH, Ulm, Germany) with 0.1 µmol sensitivity and an intra-assay CV of 2.3%. Total antioxidant capacity (TAC) levels were measured using a commercial ELISA kit (ZellBio GmbH, Ulm, Germany) with 0.1 mmol sensitivity and an intra-assay CV of 5.5%. Finally, the plasma level of carboxymethyl lysine (CML) was assessed using the ELISA method with a sensitivity of 12 ng and a commercial ELISA kit (ZellBio GmbH, Ulm, Germany) with an intra-assay CV of 1.5%.

Statistical analysis

Data were analyzed using SPSS software (Version 20.0; SPSS, Chicago, IL). We assessed the normality of variables using the Shapiro–Wilk test. Means ± SD and numbers (percentages) were used to report results for quantitative and qualitative variables, respectively. We used the Chi-square analysis to compare possible differences between the ZJ and control groups in qualitative variables. Quantitative variables with normal distribution were compared between ZJ and control groups using the independent two-sample *t* test. Within-group differences in mean values of quantitative variables were assessed using the paired *t* test. We used the analysis of covariance (ANCOVA) to assess the possible difference in post-intervention values of parameters while controlling for differences in the baseline values. All final analyses were performed using intention-to-treat (ITT) analyses, including all enrolled and randomized participants. In this method, we used multiple imputation methods to impute the missing values for biochemical variables. We used the observed (baseline) variables to impute the missing outcome value using a linear regression model.

Results

Out of the 48 patients with T2D who were randomly assigned to the study groups (24 in the ZJ group and 24 in the control group), 8 patients (4 from each group) did not complete the 12-week follow-up due to lack of cooperation (refusal to continue due to COVID-19 infection) or travel [see Figure 1]. Therefore, 20 participants from each group, aged 30-65 years, completed the 12-week follow-up. Data analyses were performed using the ITT analysis method for all 48 enrolled and randomized participants. The compliance rate among participants was more than 90%, and no specific complaints or adverse effects were reported for participants based on the visits during follow-up of study (at the 6th and 12th weeks of the study).

According to the findings in Table 1, the mean ± SD age of all participants (47.9% men) was 57.19 ± 11.08 years. The mean ± SD BMI in the intervention and control groups

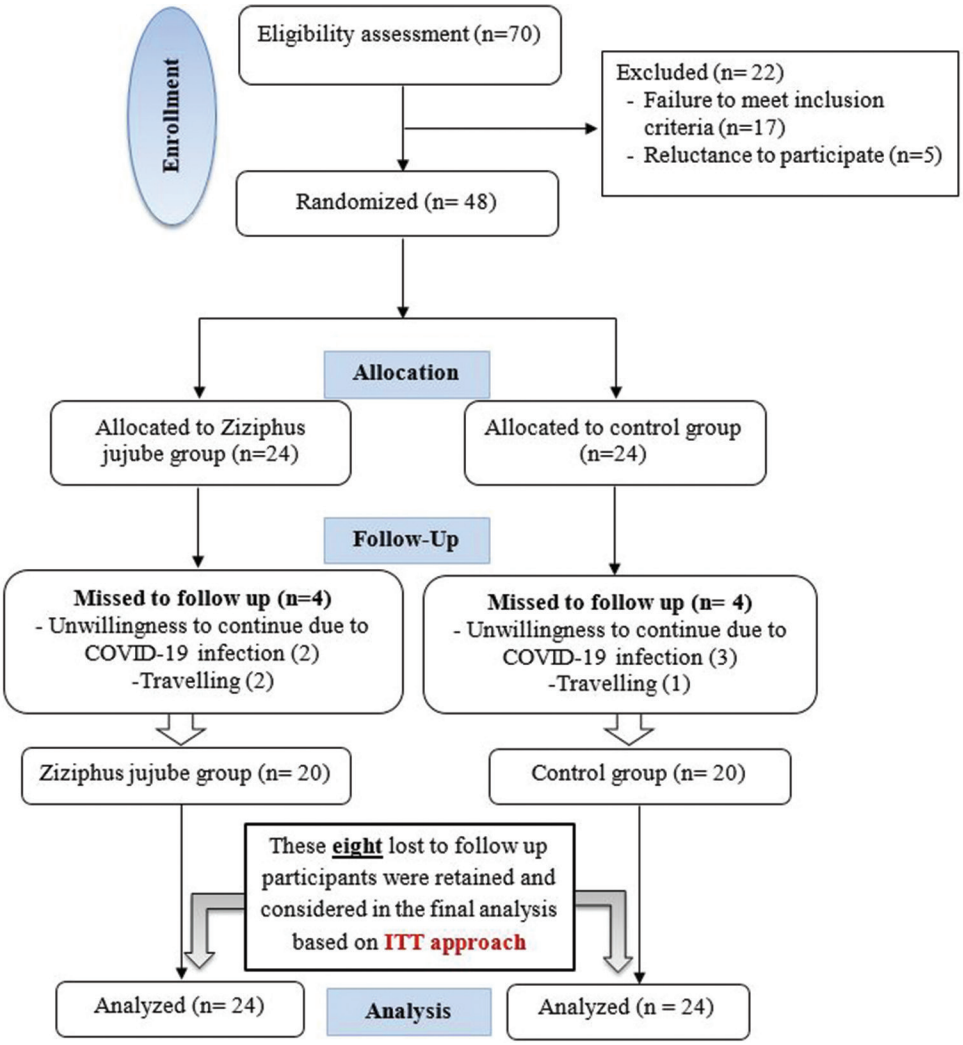

Figure 1: The CONSORT flow diagram of the current study

was 30.81 ± 4.37 and 29.87 ± 4.08 kg/m^2, respectively. Our results showed that the baseline characteristics of the patients, including age, sex, weight, BMI, educational level, duration of diabetes, and PA, did not differ between the ZJ and control groups. Table 2 indicates that the intakes of energy, carbohydrate, protein, total fats, fiber, vitamin E, vitamin C, potassium, magnesium, selenium, and calcium did not differ between the ZJ and control groups at the beginning and after the 12-week intervention. There were no significant changes in energy and nutrient intake within each group after the 12-week follow-up.

The between-group comparisons using ANCOVA analysis [Table 3] indicate that after adjusting for baseline values, a significant decrease in fasting plasma insulin level (up to 4.6 mIU/L, $P = 0.034$) and HOMA-IR ($P = 0.003$) was observed in the ZJ group compared to the control group ($P < 0.05$). We also observed a significant decrease in SBP (up to 7 mmHg) and DBP (up to 5 mmHg) in the ZJ group compared to the control group at the end of week 12 ($P < 0.05$). Although there was a significant reduction in plasma CML concentrations in the ZJ group compared to baseline ($P < 0.001$), this change was not significant when compared to the control group [$P = 0.441$; Table 3]. In addition, significant changes were observed in the plasma levels of MDA and TAC within each group during the study. However, the reductions in MDA ($P = 0.760$) and TAC ($P = 0.548$) levels during the 12-week follow-up were not significantly different between the ZJ and control groups [Table 3].

Discussion

In the current study, we examined the possible effects of dried ZJ fruit consumption on various cardiometabolic factors, including insulin-related indices, blood pressure, oxidative stress, and AGEs production in T2D patients with excess weight. The ZJ group showed reductions in mean insulin level, HOMA-IR, SBP, and DBP compared to the control groups. However, no significant differences were observed between the ZJ and control groups in mean MDA, TAC, and CML changes. Our findings also revealed that the nutrients and energy intakes were similar between

Table 1: Baseline data of T2D patients in the intervention and the control groups

Variables[a]	Study groups		*P*
	Ziziphus jujube (*n*=24)	Control (*n*=24)	
Age (Years)	57.33±10.52	57.04±11.83	0.739
Males *n* (%)	11 (45.8)	12 (50.0)	0.565
History of type 2 diabetes (Years)	4.75±3.01	4.67±2.85	0.950
Smoking *n* (%)	1 (4.2)	1 (4.2)	0.584
Physical Activity (MET minutes/week)	1138.0 (940.0)	1500.6 (1076.9)	0.221
Education Level *n* (%)			
<Diploma	11 (45.8)	12 (50.0)	0.571
≥Diploma	13 (54.2)	12 (50.0)	
Weight (kg)	83.42±14.51	83.44±14.00	0.995
BMI (kg/m^2)	30.81±4.37	29.87±4.08	0.485
FPG (mmol/L)	8.80±2.07	8.14±2.05	0.271
TGs (mmol/L)	2.14±0.65	1.82±0.83	0.162
TC (mmol/L)	4.69±1.22	4.15±0.81	0.080

[a]Continuous variables were expressed as mean±SD. Categorical variables were reported as *n* (%). *P* value was determined based on an Independent two-sample *t*-test and Chi-square for continuous variables and categorical variables, respectively. BMI: Body mass index; FPG: Fasting plasma glucose; TGs: Triglycerides; and TC: Total cholesterol

the two groups, suggesting that dietary variables did not confound the study results.

Dysglycemia and disorders related to insulin metabolism are common metabolic abnormalities in T2D patients, particularly those who are obese or overweight.[25] Despite the use of various pharmaceutical agents, dietary interventions, and lifestyle modifications, many T2D patients struggle with poor glycemic control, which can lead to life-threatening vascular complications.[11,25] As a result, herbal medicine has gained attention as a complementary and alternative treatment for improving glycemic control in diabetic patients.[10,26,27] In the current study, consuming 30 g/day of dried ZJ fruit for 12 weeks resulted in a significant decrease in plasma insulin levels and HOMA-IR in diabetic patients. To our knowledge, only a few clinical trials have assessed the effect of ZJ on glycemic control indices, and their results have been inconsistent.[18,19] A trial conducted in Isfahan City reported a significant effect of ZJ intake on HbA1c level; however, it did not find significant reductions in fasting blood glucose or 2-hour blood glucose levels.[18] In another study by Irannejad *et al*.,[19] 30 g/day of ZJ reduced plasma insulin levels and HOMA-IR in diabetic patients; however, no significant effect of ZJ intake on plasma glucose was reported.

Overall, the results of the current study, along with findings from previous research, suggest a potentially beneficial effect of ZJ intake on glycemic indices in T2D patients, although results remain somewhat inconsistent. Some factors may explain this variability. For instance, differences in baseline levels of glycemic indices, such as insulin levels, among the trials could contribute to the inconsistent findings regarding the effectiveness of ZJ on glycemic control. Moreover, variations in participants' metabolic and clinical conditions at the baseline of the studies may have influenced the conflicting results. Notably, participants in the current study have higher excess weight and elevated blood pressure compared to the population of two previous trials at baseline. Therefore, it can be expected that the intake of ZJ might have a more pronounced impact on cardiometabolic indicators in diabetic patients with more unstable metabolic conditions. Furthermore, discrepancies in study results could also arise from variations in the control of study conditions during the intervention period, including adherence to ZJ consumption and potential changes in participants' lifestyle factors, such as diet and physical activity, during the follow-up period.

Some previous reports suggest that ZJ consumption may have beneficial effects on insulin resistance (IR), metabolic syndrome (MetS), and T2D, due to its rich polyphenol and other bioactive compound contents.[15,26,28] ZJ fruit is rich in christinin-A, an important saponin glycoside that can improve glucose utilization in diabetic rats.[26] Another potential mechanism by which ZJ may benefit glycemic control is its high vitamin A content. Vitamin A can enhance insulin sensitivity via activation of insulin receptors and protein tyrosine phosphatase 1B.[29,30] Moreover, according to an experimental study, a higher intake of dried ZJ fruit reduced dyslipidemia and IR by activating the IRS-1/PI3K/Akt pathway.[28] Furthermore, higher ZJ intake has been associated with improved hepatic function, which may lead to increased plasma glucose uptake and its subsequent utilization.[15]

Elevated blood pressure is a common complication among T2D patients,[31] and effective blood pressure management can reduce cardiovascular risk in this population.[32] In our study, dried ZJ intake showed a therapeutic effect on both SBP and DBP in T2D patients. In a study by Irannejad Niri *et al*.,[19] administrating 30 grams of Ziziphus Vulgaris resulted in a significant reduction in SBP and DBP compared to baseline measurements in the intervention group;

Table 2: Nutritional characteristics of patients in the intervention and the control group at baseline and end of 12-week follow-up of study

Dietary variables	Study groups		*P**
	Ziziphus jujube (*n*=24) Mean±SD	Control (*n*=24) Mean±SD	
Energy (Kcal)			
Baseline	1672.8±92.2	1664.1±97.2	0.752
End of intervention	1610.8±96.4	1603.4±77.6	0.772
Carbohydrate (% energy)			
Baseline	56.64±2.73	54.23±3.79	0.147
End of intervention	55.47±2.94	54.82±3.40	0.484
Protein (% energy)			
Baseline	15.14±1.45	14.73±1.54	0.349
End of intervention	14.97±1.61	14.55±1.50	0.354
Fat (% energy)			
Baseline	29.21±2.70	31.02±3.00	0.250
End of intervention	29.54±2.21	30.61±2.70	0.141
Fiber (g/day)			
Baseline	16.3±1.3	15.8±1.2	0.265
End of intervention	16.0±1.2	15.5±1.0	0.201
Vitamin E (mg/day)			
Baseline	8.26±1.25	8.60±1.38	0.408
End of intervention	7.77±1.28	7.86±1.23	0.809
Vitamin C (mg/day)			
Baseline	72.92±6.29	74.03±6.00	0.556
End of intervention	80.23±6.96	73.18±5.67	0.001
Calcium (mg/day)			
Baseline	403.98±64.14	418.53±55.21	0.467
End of intervention	399.60±63.61	412.23±55.31	0.826
Potassium (mg/day)			
Baseline	2120.33±162.23	2161.59±189.68	0.447
End of intervention	2127.28±163.45	2140.25±188.43	0.810
Selenium (mcg/day)			
Baseline	403.98±64.14	418.53±55.21	0.467
End of intervention	399.60±63.61	412.23±55.31	0.826
Sodium (mg/day)			
Baseline	1898.84±118.81	1918.00±93.16	0.561
End of intervention	1886.24±110.33	1902.10±94.44	0.616

Results are reported as mean±SD. **P* value was determined based on the independent two-sample *t*-test

however, this reduction was not statistically significant when compared to the control group. Although both studies reported similar clinical effects of ZJ consumption on blood pressure control, the effect observed in our study was more pronounced, likely because all participants were hypertensive or pre-hypertensive at baseline. An experimental study has reported a potential protective effect of long-term intake of ZJ on SBP, DBP, and mean arterial pressure.[17] It has been suggested that the higher content of bioactive compounds such as flavonoids, alkaloids, terpenoids, phenols, and antioxidant vitamins in ZJ fruit may play a key role in cardiovascular health and blood pressure regulation by enhancing nitric oxide production in the endothelial cells.[17,33] Additionally, certain active compounds in ZJ fruit, such as sapogenin, betulinic acid, and jujuboside, may help reduce blood pressure by modulating vascular tone and activating the endothelial NOS system.[34-36]

Dietary AGEs are inevitable byproducts of improper macronutrient metabolism in T2D patients. Reactive oxygen species (ROS) and AGEs contribute to diabetic vascular complications, especially in diabetic patients with poor glycemic control.[37] In the present clinical trial, ZJ consumption did not significantly affect oxidative stress markers, including plasma MDA and TAC concentration. In agreement with our results, a previous clinical trial also found that the ZJ consumption had no beneficial effects on serum levels of MDA and TAC.[18] These findings suggest that the antioxidant compounds in 30 g ZJ fruit are insufficient to improve the oxidative status in T2D patients. However, consuming ZJ and other antioxidant-rich fruits can help reduce the production of oxidative compounds.[10,16] In addition, in our study, more than 50% of patients had diabetes for 1 to 3 years, indicating that they were likely not in critical oxidative stress conditions.

Table 3: Plasma concentrations of cardiometabolic markers, oxidative stress indices, and carboxymethyl lysine levels in the intervention and the control group

Biochemical variables	Study groups				P^a
	Ziziphus jujube (*n*=24)		Control (*n*=24)		
	Mean±SD	Chang %	Mean±SD	Chang %	
Fasting plasma insulin (mIU/L)					0.034
Baseline	14.9±6.7	-30.87	12.1±7.4	0.82	
End of intervention	10.3±4.2		12.2±4.0		
P^b	0.005		0.932		
HOMA-IR					0.003
Baseline	5.82±2.98	-37.80	4.60±3.32	10.43	
End of intervention	3.62±1.80		5.08±2.86		
P^b	0.003		0.380		
Carboxymethyl lysine (ng/ml)					0.441
Baseline	332.0±78.6	-23.70	308.2±109.0	-27.31	
End of intervention	253.2±94.3		224.0±88.7		
P^b	<0.001		<0.001		
Total antioxidant capacity (mM)					0.548
Baseline	1.29±0.17	34.88	1.25±0.17	32.80	
End of intervention	1.74±0.26		1.66±0.30		
P^b	<0.001		<0.001		
Malondialdehyde (umol/l)					0.760
Baseline	5.52±2.09	-25.00	5.19±2.05	-20.61	
End of intervention	4.14±1.46		4.12±1.72		
P^b	0.003		0.003		
Systolic blood pressure (mmHg)					<0.001
Baseline	137.5±4.0	-5.38	135.5±2.6	-0.95	
End of intervention	130.1±4.6		134.2±2.6		
P^b	<0.001		0.078		
Diastolic blood pressure (mmHg)					<0.001
Baseline	89.7±4.0	-5.68	88.8±3.9	-1.12	
End of intervention	84.6±3.1		87.8±2.9		
P^b	<0.001		0.069		

[a]The ANCOVA test was used to calculate the between-group *P*, which was adjusted for baseline measures. [b]The paired sample *t*-test was used to determine the within-group *P*

Therefore, it is not surprising that ZJ fruit consumption did not significantly reduce plasma MDA and TAC levels in our T2D participants.

This study is the first to focus on the effect of dried ZJ intake on CML, as a marker of AGEs in T2D. Although the intervention group showed a significant within-group reduction in CML, this change was not significantly different from the control group. While no significant results were observed regarding AGEs in this study, our findings lay the groundwork for future research. Further studies should examine the effects of varying amounts of ZJ fruit or its extracted compounds on AGEs levels in populations with different characteristics.

Strengths and limitations

The current study had several strengths. It is the first to examine the effects of ZJ intake on oxidative stress indicators and CML, an indicator of AGEs products in T2D patients with high BMI and elevated blood pressure. Also, our study demonstrated high completion rates, appropriate design, and strong adherence to the ZJ administration protocol. We also accounted for potential confounding factors such as nutritional variables, physical activity, BMI, and smoking and found no significant differences between the ZJ and control groups in these variables. However, our study has some limitations. The relatively small sample size may have reduced the power to detect effects of ZJ on certain indicators, such as oxidative stress-related variables. Another limitation was the inability to administer a placebo in the control group. Despite this, we used block randomization and closely monitored participant compliance with the study protocol in both the intervention and control groups to minimize biases and control for confounding factors. It is important to note that block randomization was performed by an independent statistician, and the allocation sequence was concealed from both the investigators and participants until the completion of the statistical analysis. Based on our results, energy and

macronutrients intakes did not differ between the two groups, suggesting that dietary factors did not confound our analysis. While assessing changes in additional AGEs indicators, such as fructosamine levels, could have provided a more comprehensive evaluation of the effects of ZJ in T2D, financial constraints limited our ability to conduct these measurements. Furthermore, due to the rapid spread of the COVID-19 infection within the Iranian population and the increased vulnerability of diabetic patients to this infectious disease, some selected participants showed reduced cooperation. Despite these challenges, we utilized the ITT approach to analyze the data of all enrolled and randomized participants based on the multiple imputation method, and by imputing missing values for biochemical variables.

Conclusions

In conclusion, our study suggested that administrating 30 g/day of dried ZJ fruit to T2D patients for 12 weeks can be beneficial in improving some cardiometabolic markers, including plasma insulin, HOMA-IR, and blood pressure. However, ZJ consumption did not significantly reduce oxidative stress or AGEs products in T2D patients. Further research is recommended to assess and confirm the metabolic mechanisms underlying the hypoglycemic and antihypertensive effects of ZJ in T2D patients with excess weight.

Ethical approval and consent to participate

The protocol study for our research was reviewed and approved by the ethics research committee of the Research Institute for Endocrine Sciences at the Shahid Beheshti University of Medical Sciences, Tehran, Iran, with the identification code: IR.SBMU.ENDOCRINE.REC.1397.137. Our study follows the ethical standards outlined in the Declaration of Helsinki. We obtained written informed consent from all participating patients.

Acknowledgments

We would like to express our gratitude to the volunteers who participated in our study and the staff of the Endocrinology Clinic in the Hospital of Ayatollah Taleghani, Tehran, Iran, for their valuable cooperation and assistance. We would also like to extend our appreciation to the research laboratory staff of the Research Institute for Endocrine Sciences for their technical support and assistance.

Authors' contributions

H.F., F.A., **P.M.**, and **G.A.** designed the study and conceptualized the manuscript. **H.F.**, **F.T.**, **Sh. S.,** and **M.H.** contributed to the manuscript's research, data analysis, and interpretation. **H.F., P.M., and M.KJ** wrote and corrected the manuscript. Also, **P.M.** and **F.A.** supervised the project. All authors read and approved the final manuscript.

Financial support and sponsorship

This work was funded by the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Conflicts of interest

There are no conflicts of interest.

Received: 13 May 24 **Accepted:** 18 Dec 24
Published: 26 Aug 25

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