Effects of traditional fermented food (Ash-Kardeh) consumption on blood glucose, blood pressure, and lipid profile in type 2 diabetes: a study protocol for a randomized clinical trial

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Abstract

Background Many therapeutic methods are recommended for the management of Type 2 diabetes. Ash-Kardeh contains several components such as flavonoids, antioxidants, and dietary fiber which can affect lipid profile and blood pressure in diabetic patients. However, no study has examined the effects of Ash-Kardeh consumption on blood glucose, blood pressure, and lipid profile in type 2 diabetic patients; therefore, the aim of this study will be to examine the effects of Ash-Kardeh consumption on blood glucose, blood pressure, and lipid profile in type 2 diabetic patients.

Methods This study is a randomized, no-blinded, controlled clinical trial in which 44 type 2 diabetic patients will be randomly allocated to intervention and control groups. Individuals in both the intervention and control groups will receive (the usual treatment of diabetic patients) for 6 weeks, while those in the intervention group will receive (250 g of traditional fermented food daily in addition to the usual treatment) at the same time. Assessment of anthropometric measures, blood pressure, and biochemical parameters including serum concentrations of fasting blood sugar, high-density lipoproteins-cholesterol, low-density lipoproteins-cholesterol, total cholesterol, and triglyceride will be performed at the study baseline and end of the trial.

Keywords Type 2 diabetes · Hypertension · Dyslipidemias · Fermented food

Abbreviations

A-K	Ash-Kardeh
BMI	Body mass index
FBS	Fasting Blood Sugar
HDL-C	High-density lipoproteins-cholesterol
LDL-C	Low-density lipoproteins-cholesterol
T2D	Type 2 Diabetes

TC	Total cholesterol
TG	Triglyceride

Trial registration This clinical study was registered on the <https://fa.ict.ir> website (shortened name ETFFT2D; registration number IRCT20170202032367N3).

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Background

Diabetes is among the most common chronic metabolic disorders, sometimes referred to as the “silent epidemic”, and is a major global health problem, with numerous and dangerous side effects such as atherosclerosis, retinopathy, nephropathy, and peripheral neuropathy with a risk of diabetic foot complications leading to a decline in patients’ quality of life and ultimately death [1, 2]. According to reliable statistics, the global prevalence of diabetes in the 18–99 years old population group was 8.4% in 2017 and reaches 629 million by 2045. In Iran, the prevalence of diabetes in the 25–64 years old population group is 7.7% [3, 4]. There are generally 2 types of diabetes: Type 1 and 2. Type 2 Diabetes (T2D) accounts for about 90–95% of diabetic patients [5]. In T2D, insulin resistance or insufficient insulin secretion from beta cells in the pancreas causes high blood sugar and various complications [6]. Currently, the most important treatment for T2D is glycemic control, achieved through drug therapy with various types of hypoglycemic drugs; In contrast, hypoglycemia, gastrointestinal problems, weight gain, and heart failure are among the most significant problems associated with the use of these drugs [7].

Numerous studies have been conducted toward seeking a suitable strategy to control T2D and reduce its associated complications. Some of them have shown that diet and medical nutrition therapy play an important role in controlling blood glucose in diabetic patients, especially T2D, while dietary interventions do not have medication side effects [8, 9]. Plant-based diets, as well as fermented foods, have received a great deal of attention in improving T2D. In addition to blood glucose, blood lipids can also be affected by nutrients in these diets [10–12]. One of the components of the plant-based diet is the Kardeh plant from the Araceae family [13]. The Araceae family contains flavonoids, anthocyanins, amines, and saponins. They all have potent antioxidant properties to remove free radicals [14, 15]. One of the most important compounds found in the Kardeh plant is flavonoids. Due to their antioxidant properties, they lead to lower blood sugar concentrations in animal samples [14, 16]. A traditional fermented food called Ash-Kardeh (A-K) is produced from grains and Kardeh plant. It is notable the bacteria in A-K are primarily from the lactic acid bacteria family [17]; therefore, it can have probiotic, prebiotic, or synbiotic properties [18, 19].

The beneficial effects of A-K consumption on blood glucose concentration might be explained by its dietary fiber content [12]. A possible mechanism that may lead to an improved lipid profile in type 2 diabetic patients through the consumption of A-K in its probiotic properties [17–19]. Also, during the fermentation process by lactic acid bacteria, numerous compounds with biological properties are obtained, such as functional peptides that can lower blood cholesterol and blood pressure via their antioxidant properties; therefore,

one of the other possible mechanisms of A-K may depend on the compounds obtained from the fermentation process [20].

According to some traditional sources, A-K can be used to treat diseases such as hyperlipidemia, hypertension, infection, diabetes, and jaundice [18, 21]; however, we are aware of no study that has examined the beneficial and therapeutic effects of A-K consumption on human health; Therefore, the current study will be conducted to examine the effects of A-K consumption on blood glucose, lipid profile, and blood pressure in type 2 diabetic patients.

Material and methods

Participants

This is a randomized clinical trial that will be done in Yasuj, Iran, in 2019–2020. Patients with T2D aged 30–65 will be recruited from health centers affiliated with Yasuj University of Medical Sciences, Yasuj, Iran. T2D will be confirmed by an endocrinologist or based on criteria stipulated by the American Diabetes Association: Fasting Blood Sugar (FBS), ≥ 126 mg/dl; 2-h PG, ≥ 200 mg/dl during OGTT; A1C, $\geq 6.5\%$; random plasma glucose, ≥ 200 mg/dl [22]. In addition to inclusion criteria, we will invite type 2 diabetic patients who consume their medications regularly and on a timely basis and have suitable control to participate in the study.

Exclusion criteria

We will exclude those with Body Mass Index (BMI) greater than 35 kg/m^2 ; smokers; those on weight loss or diet programs for the past 6 months; alcoholic beverage consumers; those who take any probiotic and prebiotic products or supplements for 6 months prior and during the intervention; pregnant and lactating women; those who require commencing antibiotic and immunosuppressive drugs or estrogen therapy during intervention; patients suffering from hypoglycemia complications associated with T2D; subjects with gastrointestinal problems and short bowel syndrome; individuals with kidney, liver, pulmonary or parathyroid diseases, asthma, and allergies.

Informed consent form process

All participants will enter the study with informed prior consent. The ethics committee of Tehran University of Medical Sciences approved the study as well as the written consent in Persian (Appendix A) that will be obtained from all participants. The trial was registered at the Iranian website (www.irct.ir) for registration of clinical trials as IRCT20170202032367N3. The written consent, study objectives, methods, benefits, and risks will be fully explained to participants and will be signed by them before

enrollment into the study. All participants will be notified that study participation will be voluntary and that they could withdraw at any time. The subjects will be assured that their reluctance to participate in the study does not affect their routine normal health care at health centers and clinics. The participants will be given sufficient time to read the consent form and ask any questions. After the participants sign the consent form, a copy of the document will be given to the participants.

Sample size calculation

Considering the mean and standard deviation of Total Cholesterol (TC) changes in the intervention (-0.2 ± 0.33) and control (0.18 ± 0.51) groups, we calculated the required sample size using the following formula:

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 \times (S_1^2 + S_2^2)}{(\mu_1 - \mu_2)^2}.$$

The sample size was calculated to be 20 persons for each group. Taking into account the 10% drop-out in each group during follow-up, we determined a sample size of 22 persons for each group [23, 24].

Study design and intervention

In this study, type 2 diabetic patients who visit health centers in Yasuj will be invited to participate. After recruiting patients, participants will be stratified based on age (30 to 50 and 50 to 65 years), BMI (18.5 to 24.9 and 25 to 35), and the disease duration (6 months to 9 years and 10 to 20 years) into different blocks, and for each patient in a certain block, a matched person in terms of mentioned variables will be placed in that block. Then, the two patients in a given block will be randomly allocated to the intervention ($n = 22$) or control ($n = 22$) groups using a computerized randomization system. To conduct randomization, an identification code will be given to each diabetic patient and hence, the code of patients with the same age, BMI, and duration of the disease will be entered into the computer's randomization software. Ultimately, patients with the same conditions will be randomly assigned to the intervention or control groups. Random allocation will be performed by a person unaffiliated with the study. Individuals in both the intervention and control groups will receive (the usual treatment of diabetic patients) for 6 weeks, while those in the intervention group will receive (250 g of traditional fermented food daily in addition to the usual treatment) simultaneously. The assessment of variables will be performed at the study baseline and end of the trial (Table 1).

After assigning the patients to either the intervention or control groups, since there will not be blinding in this study, a face-to-face meeting will be held with the members of each group, during which participants will be asked not to alter their routine physical activity, their type/dosage of medications, or usual dietary intakes throughout the study and not to consume any supplements other than the ones provided to them by the investigators. Moreover, they will be told not to consume other fermented foods and processed foods during the study. The physical activity level will be evaluated by a short form Physical Activity Questionnaire (IPAQ) (Appendix B). Participants will be asked to report the time they spent on their physical activity during the week, at the study baseline. To evaluate the patients' nutritional intake and to assure the consumption of traditional fermented food, the dietary intake of participants will be assessed weekly using 3-day dietary records (Appendix C) throughout the intervention. The dietary records will be based on estimated values in household measurements. The 3-day diet history checklist is a one-page checklist, recording the food dietary intake of patients over the past 24 h. Nutritionist IV software will be deployed to obtain nutrient intakes of participants based on these 3-day food records.

Treatment of subjects

A-K or Hara is a traditional fermented food that is produced from cereals and plants. The process of producing A-K is initiated by microorganisms that are mainly part of the family of lactic acid bacteria and are present in raw materials with which A-K is produced. The method of cooking A-K is to grind the Kardeh plant and rice and then add a little starter (a little flour with buttermilk or yogurt) to the mixture; then transfer all the ingredients to a baking pan and add a little water. In the next step, the pan is kept in an environment with a temperature of about 45 °C for 24 h to perform the fermentation process, and finally, the contents of the baking pan are cooked to obtain A-K [17, 25]. Quality control of the traditional fermented food (A-K) will be performed under the supervision of a Food and Drug Administration employee in Yasuj, Iran. During the intervention, the amount of A-K that will be needed for 1 week will be poured into bottles for each participant (Table 1). A standard Bowl (which contained 250-g A-K) will be given to Intervention group participants and all will be asked to use the same Bowl to make sure that the daily portion they are consuming is the one investigators requested. At the end of each week, when we meet with participants to deliver A-K bottles, we will ask them about A-K consumption and following instructions given to them. Participants will be asked to keep the bottles in the refrigerator and consume 250 g of the bottle content daily. During the intervention, individuals in the control group will receive (the usual treatment of diabetic patients) for 6 weeks; although no other intervention will

Table. 1 Product delivery program and Follow up participants

Visit time	meeting day (day 0)	Week 1 (day 7)	Week 2 (day 14)	Week 3 (day 21)	Week 4 (day 28)	Week 5 (day 35)	Week 6 (day 42)	Week 7 (day 49)
Delivery of A-K	*	*	*	*	*	*		
Anthropometric measurements	*						*	
Blood pressure measurement	*						*	
Blood sampling	*						*	
record 24-h food recall	*	*	*	*	*	*	*	
End of intervention							*	
Evaluation of adverse events	*	*	*	*	*	*	*	*

be considered for patients in the control group at this period, after the intervention, a standard Bowl and the amount of A-K that will be needed for 1 week will be given to control group participants too. The amount of ingredients in the 250 g of A-K is delineated in (Table 2).

Product intended use

A-K will be used as an oral snack in the evening meal, (should be consumed on its own).

Product acquisition

A-K will be prepared and distributed by the study executive team at no cost to the participants.

Assessment of variables

The Demographic Information Questionnaire will be used to obtain information on age, gender, anthropometric measures, duration of diabetes, level of physical activity, and medication consumption including hypoglycemic (such as Insulin, Metformin, Gliclazide, Acarbose, and Pioglitazone), hypolipidemic (such as Atorvastatin, Rosuvastatin, and Lovastatin), and antihypertensive drugs (such as Amlodipine, Losartan and

Atenolol). Also, primary outcomes including serum concentrations of FBS, High-Density Lipoproteins-Cholesterol (HDL-C), Low-Density Lipoproteins-Cholesterol (LDL-C), Triglyceride (TG), TC, and blood pressure will be measured at the beginning and end of the study.

Anthropometric measurements

Weight will be measured using a digital scale to the nearest 0.1 kg, without shoes and wearing minimal clothing. Height will be measured to the nearest 0.1 cm by using a non-stretch fixed tape measure (Seca), without shoes. Finally, the BMI will be calculated as weight in kilograms divided by height in meters squared.

Physical activity level evaluation

A short-form physical activity questionnaire [26], translated into Persian, will be gathered from all patients to assess physical activity for study baseline. The physical activity data will be converted to minutes per week and expressed as (MET-minute/week) for data processing. Finally, after processing the information that will be obtained from the questionnaires, the level of physical activity of the participants will be divided into three categories of low, moderate, and high physical activity, compliant with IPAQ guidelines. Furthermore, patients in both groups will be asked to not change their physical activity throughout the trial.

Blood pressure

Patients' systolic and diastolic blood pressures will be measured twice (with 15-min interval) using a mercury barometer calibrated in a sitting position. 30 min before measurement, patients will be asked to not consume tea, coffee, and cigarettes and not to engage in heavy physical activity. The mean of two consecutive steps of blood pressure measurement will be recorded as the patient's blood pressure.

Table 2 Ingredients in 250 g of traditional fermented food (Ash-Kardeh)

Energy (kcal)	40
Carbohydrate (g)	8.5
Protein (g)	1
Fat (g)	0.2
Sodium (mg)	29
Calcium (mg)	19
Potassium (mg)	104
Vitamin C (mg)	2
Vitamin B9 (μg)	9
Vitamin B12 (μg)	0
Fiber (g)	1

Biochemical assessment

After 12 h of overnight fasting, a 5 mL venous blood sample will be taken from each patient at the study baseline, and after a 6-week intervention. Blood samples will be immediately centrifuged to separate serum. Serum concentrations of FBS, TG, TC, and HDL-C will be determined using commercial kits (Pars Azmun, Tehran, Iran). All TC, HDL-C, and TG will be measured by autoanalyzer. The Friedewald formula will be used to calculate LDL-C when serum TG level was <400 mg/dl. The Friedewald formula: $(LDL-C = TC - (HDL-C + [TG/5]))$. FBS will be measured by Glucose kit with enzymatic and Colorimetric method (glucose oxidase).

Adverse event reporting

Any adverse reactions should be reported to the study executive team immediately. In the case of adverse events, they will be recorded by the study executive team.

Statistical methods

The Kolmogorov–Smirnov test will be performed to examine the normal distribution of variables. For the non-normally distributed data, log transformation will be utilized. A Chi-square test will be used to examine differences in qualitative variables between intervention and control groups. The independent-sample t-test will be used to detect differences in quantitative variables between intervention and control groups. Also, the mentioned test will be used to compare between-group changes in outcome variables. To implement the within-group comparison, we will utilize the paired-sample t-test. Multivariate analysis of covariance (ANCOVA), will be used to examine the effects of A-K consumption on outcome variables. The baseline value of outcome variables and potential confounding variables which are different between intervention and control groups will be adjusted in (ANCOVA) analysis to avoid the potential risk of bias and detect independent results. The value $P < 0.05$ will be considered as a statistically significant level. All statistical analyses will be conducted using SPSS, version 22 (SPSS Inc).

Limitations of this study

We will not investigate various dose-dependent effects of A-K consumption. Furthermore, liver function tests will not be evaluated to assess the effects of this fermented food on the liver. Despite using validated questionnaires for assessment of physical activity level and dietary

intake, misclassification of participants will not be fully excluded. Change in dietary intake of participants throughout the intervention is the most important concern for investigators; therefore, we will do our best to ensure that participants do not change their usual diet.

Trial status The recruitment phase was scheduled from October 2019 to January 2020. The intervention will be conducted for 6 weeks for each participant and after performing laboratory tests, data analysis and evaluation will be conducted. Finally, the results of this trial will be published.

Availability of data and materials Not applicable.

Compliance with ethical standards

Ethics This study was confirmed by the ethics committee of Tehran University of Medical Sciences (code number: IR.TUMS.VCR.REC.1398.462). Moreover, it was registered in the Iranian Registry of Clinical Trials www.irct.ir (code number: IRCT20170202032367N3).

Conflict of interest No conflicts of interest have been reported.

Appendix 1

Consent form

Research Project Title:
Effects of Traditional Fermented Food (Ash-Kardeh) Consumption on Blood Glucose, Blood Pressure, and Lipid Profile in Type 2 Diabetes: A Study Protocol for a Randomized Clinical Trial.

Project Executives:
Hossein Imani and Sayed Omid Salehi.

I know that the goals of this research are:

1. Effects of Traditional Fermented Food Consumption on Blood Glucose in Type 2 Diabetes.
2. Effects of Traditional Fermented Food Consumption on Blood Pressure in Type 2 Diabetes.
3. Effects of Traditional Fermented Food Consumption on Lipid Profile in Type 2 Diabetes.

I know that I voluntarily take part in this research and I do not have to participate in the research. I was assured that if I do not willing to participate in this research, I am not deprived of common therapeutic cares and I am still in contact with the clinical center and physician. Even after I agree to participate in the project, I know that I can leave the project whenever I want, after informing the executive of my leave. If I leave the project, I will not be deprived of common clinical services.

I contribute to the project in this way: I will complete questionnaires containing demographic information and evaluation of nutritional status, I will come for taking my blood sample and follow the type of intervention considering membership in one of the two groups of intervention and control. Project executives are obligated to keep my data secret and they are only allowed to release the general and collective results of the project without mentioning my name and specifications.

First name and family name of participant:

Address and telephone number:

Date and signature of participant:

I was introduced to Mr. Sayed Omid Salehi for a response to my queries. I was told to talk to him whenever problems or questions come up for guidelines.

Address and telephone number of the corresponding person:

Date and signature of the corresponding person:

Appendix 2

International physical activity questionnaire (IPAQ) – short form

First name and last name: _____ Questioner Name: _____

1. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling? Days per week

No vigorous physical activities ☐ → (Skip to question 3)

2. How much time did you usually spend doing vigorous physical activities on one of those days?

..... Hours per day Minutes per day don't know/Not sure ☐

3. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? (Do not include walking)

..... Days per week

No moderate physical activities ☐ → (Skip to question 5)

4. How much time did you usually spend doing moderate physical activities on one of those days?

..... Hours per day Minutes per day don't know/Not sure ☐

5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

..... Days per week

No walking ☐ → (Skip to question 7)

6. How much time did you usually spend walking on one of those days?

..... Hours per day Minutes per day don't know/Not sure ☐

The last question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the last 7 days, how much time did you spend sitting on a weekday?

..... Hours per day Minutes per day don't know/Not sure ☐

Appendix 3

Food recall

Days	Meals	Time	Food items	Serving	Amount (gr)
Day 1 (working day)					
Day 2 (working day)					
Day 3 (weekend)					

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