Cynara scolymus L. in Treatment of Hypercholesterolemic Type 2 Diabetic Patients: a Randomized Double-Blind Placebo-Controlled Clinical Trial

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Abstract

Background: Type 2 diabetes mellitus with hyperlipidemia is a common disease. Conventional anti-hyperglycemic and anti-hyperlipidemic drugs have limited efficacies and important side effects, so that alternative agents are needed. Previous studies suggest that fiber-free artichoke (*Cynara scolymus* L.) leaf extract may have anti-hyperglycemic and anti-hypercholesterolemic effects in hypercholesterolemic type 2 diabetic patients. Moreover, artichoke leaves are used traditionally as an anti-hyperglycemic and anti-hyperlipidemic remedy to treat diabetes mellitus and hyperlipidemia.

Objective: The effects of artichoke leaf extract in treatment of hypercholesterolemic type 2 diabetic patients were studied.

Methods: In this randomized double-blind placebo-controlled clinical trial with hypercholesterolemic type 2 diabetic patients aged 40 to 60 years not using other anti-hyperlipidemic agents and resistant to daily intake of two 5 mg glyburide tablets and two 500 mg metformin tablets, the efficacy and safety of taking fiber-free artichoke leaf extract (one 400 mg capsule t.i.d. for 2 months) combined with the aforementioned drugs in treatment of 36 patients were evaluated and compared with the placebo group (n = 36).

Results: The extract lowered the blood levels of total cholesterol and LDL cholesterol significantly (P=0.002 and p=0.040 respectively) without any significant effects on the blood levels of fasting glucose, postprandial glucose, glycosylated hemoglobin, other lipids, SGOT, SGPT and creatinine (P>0.05) compared with placebo at the endpoint. No adverse effects were reported.

Conclusion: Fiber-free artichoke extract may be a safe anti-hypercholesterolemic agent but does not improve glycemic control in hypercholesterolemic type 2 diabetic patients, suggesting the involvement of fibers in the anti-hyperglycemic effect of artichoke.

Keywords: Artichoke, Hyperlipidemia, Patient, Type 2 diabetes

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Introduction

Type 2 diabetes mellitus (T2DM) is prevalent worldwide [1]. Type 2 diabetic patients frequently have a host of other metabolic abnormalities including dyslipidemia [increased low density lipoprotein (LDL) cholesterol, very low density lipoprotein (VLDL) and triglycerides and decreased high density lipoprotein (HDL) cholesterol], vascular dysfunction, obesity and hypertension. This cluster of metabolic abnormalities, which has been termed the metabolic syndrome, is associated with a higher incidence of premature cardiovascular morbidity and mortality [2]. Multiple anti-hyperglycemic and anti-hyperlipidemic drugs with different mechanisms are often needed to achieve effective glycemic and lipid control in hyperlipidemic type 2 diabetic patients [3, 4]. Conventional anti-hyperglycemic and anti-hyperlipidemic drugs have limited efficacies and important adverse effects. Thus, more efficacious and safer anti-hyperglycemic and anti-hyperlipidemic agents are needed [5, 6]. Plants have played a significant role in maintaining human health and improving the quality of life for thousands of years [7]. Herbal supplements may be effective in prevention and treatment of diseases including cardiovascular disease [8, 9, 10]. Dried artichoke (*Cynara scolymus* L., Asteraceae family) leaves are taken traditionally at the dose of 6 g per day to treat numerous diseases including diabetes mellitus and hyperlipidemia [11]. A variety of pharmacological effects have been demonstrated for artichoke leaf extracts including inhibition of cholesterol biosynthesis in primary cultured rat hepatocytes [12] and lowering of serum cholesterol and triglycerides levels and hypercholesterolemia-induced pro-oxidant state in the liver and heart of the rats fed on high cholesterol diet [13]. Further, the artichoke leaf juice improved endothelial function, but increased the blood triglycerides level in the hyperlipidemic patients [14]. However, cynarin (the constituent of the artichoke) had no hypolipidemic effect in the patients with familial types IIa and IIb hyperlipidemias [15]. A review concluded that there is an indication that artichoke leaf extract has potential in lowering cholesterol level in hypercholesterolemic patients, the evidence is, however, as yet not convincing and also limited data exist on the safety of the artichoke leaf extract in the patients [16]. Based on the notion that dietary fibers may be beneficial in carbohydrate metabolism [17], a study found that consumption of a food supplement prepared from artichoke flowering heads for 3 consecutive months reduced fasting and postprandial glycemia, total cholesterol, LDL and triglycerides but increased HDL in hypercholesterolemic type 2 diabetic patients, however the blood glycosylated hemoglobin levels were not determined and the study was not randomized, double-blind and placebo-controlled [18]. Consistently, another study found that a meal of bread and wild artichoke (*Cynara cardunculus* L.) attenuated postprandial increase in glycemia in healthy subjects. However, the study was not double-blind and placebo-controlled [19]. A study designed to assess the ability of artichoke extract to reduce glycemia via a mechanism other than the action of its fibers found that fiber-free extract of artichoke flowering heads had hypoglycemic effect in normal and obese rats [20]. As far as is known after searching various data bases, no clinical trial concerning

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the effects of fiber-free extract of artichoke in treatment of diabetic patients has been conducted. Thus, the efficacy and safety of fiber-free extract of artichoke leaves in treatment of hypercholesterolemic type 2 diabetic patients were evaluated in the present trial.

Materials and Methods

Artichoke

Artichoke was collected from the lands in the Alborz province of Iran in August and its identity was authenticated by a botanist (Y. Ajani). A voucher specimen of the plant (number 711) has been deposited in the Central Herbarium of the Research Institute of Medicinal Plants affiliated with the ACECR (Iranian Academic Center for Education, Culture and Research). The leaves were separated from the plant, washed and dried in shade at room temperature. The dry leaves were ground into powder.

Preparation of the fiber-free artichoke extract for patients use

The dry artichoke leaf powder (20 Kg) was extracted with ethanol/water (70/30) as the solvent in a percolator for 72 h, the solvent was completely removed from the extract in a rotary evaporator, toast powder as an excipient was added to and mixed with the concentrated extract and the mixture was ground to a powder. Toast powder was used as an excipient, because with a smaller amount of it compared to other excipients, a fine dry powder of the extract could be produced. The quantity of the extract powder produced was 4.9 Kg. The excipient constituted 18.4 percent of the extract powder.

Preparation of the extract and placebo capsules

The extract powder as the drug and toast powder as the placebo were separately filled into oral gelatin capsules with identical appearance by using a hand-operated capsule-filling machine (Scientific Instruments and Technology Corporation, USA). The artichoke capsules contained 400 mg of the extract powder. Toast powder was chosen as the placebo, because it was used as the excipient in preparation of the extract powder and its appearance was relatively similar to the extract powder.

Standardization of the extract

The extract was standardized through determining the total phenolics content.

Preparation of the extract powder for spectrophotometric analysis:

The extract powder was prepared according to the method described previously [21]. About 5 g of the extract was reconstituted in 50 ml methanol using an ultrasonic bath for 2 h and left at room temperature (25 ºC) for 24 h. The sample was then filtered through Whatman no. 1 paper in a Buchner funnel. The filtered solution was evaporated under reduced pressure by a rotary evaporator at the temperature lower than 40 ºC under vacuum up to a constant weight and then dissolved in methanol. The solution was stored at -18 ºC until use.

Determination of total phenolics content:

The total phenolics content was measured through the method described previously [22] with some modification. Briefly, an aliquot (1 ml) of appropriately diluted extract or standard

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solutions of gallic acid in water (50, 100, 150, 200 and 250 µg/ml) was added to a 25 ml volumetric flask containing 9 ml of ddH_2O (double-distilled water). A reagent blank using ddH_2O was prepared. 1 ml of Folin & Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added with mixing. The solution was then immediately diluted to volume (25 ml) with ddH_2O and mixed thoroughly. After incubation for 90 min at 23 ºC, the absorbance versus prepared blank was determined at 750 nm. The total phenolics content of the extract was expressed as milligrams of gallic acid equivalents per gram of the extract. All samples were analyzed in 3 replications.

Patients

Inclusion criteria: Iranian male and female type 2 diabetic outpatients aged 40 to 60 years; patients with fasting serum glucose levels between 150 mg/dl to 200 mg/dl and blood HbA1c levels between 7% to 9% despite taking two 5 mg glyburide tablets and two 500 mg metformin tablets every day; patients using two 5 mg glyburide tablets and two 500 mg metformin tablets every day; patients with fasting serum LDL levels between 100 mg/dl to 150 mg/dl.

Exclusion criteria: Patients taking other anti-hyperglycemic and anti-hyperlipidemic agents; patients receiving insulin therapy; patients with cardiac, renal, hepatic, hematological diseases, hypothyroidism, tachycardia, vertigo and seizure; patients with a history of gallstones or gall bladder surgery; patients using estrogen, steroid, beta-blocker and thiazide; pregnant women; women planning pregnancy; breast-feeding women.

Protocol

Seventy two Iranian male and female outpatients who were eligible according to the inclusion and exclusion criteria completed this study. The demographic data of the subjects are given in the table 1. A group of thirty six patients took the artichoke extract capsules at the dose of one 400 mg capsule every 8 hours by the oral rout for 2 months and another concurrently parallel group of thirty six patients took the placebo capsules orally every 8 hours for 2 months. The dosage of the artichoke extract was based on the average dose of artichoke leaves in the traditional medicine (6 g per day) and the yield of the extraction process used in this study (20 percent). Block randomization was used for treatment allocation. The study was double-blind. Further, the patients were recommended to restrict intake of carbohydrates and fatty foods such as rice, confectionery, red meat, pies, cream pies, eggs and fatty dairy products from two months before the beginning of the trial onward. All the subjects recorded the names and amounts of the daily consumed foods for 3 days every week. To monitor the patients' compliance with the allocated treatments, the patients returned any capsules left and were asked questions about taking the capsules on their monthly visit. The treatment, diet and physical activity of the patients remained unchanged throughout the study. At the beginning and also the end of the study, the blood levels of 2 h postprandial glucose and fasting (after fasting for 12 hours) glucose, HbA1c, triglycerides, total cholesterol, LDL, HDL, creatinine and liver enzymes including SGOT and SGPT in the artichoke and placebo groups were determined with standard enzymatic kits produced by the Pars Azmoon

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company (Tehran, Iran) and an auto analyzer (Hitachi 902, Japan). The fasting glucose, HbA1c and LDL levels were the primary outcome variables. The other blood parameter levels were the secondary outcome variables. The baseline homogeneity of the blood parameter means across the artichoke and placebo groups was analyzed by the Mann-Whitney U test and P values below 0.05 were considered as significant. At the end of the study, the data of the patients in the artichoke and placebo groups were compared by the Mann-Whitney U test and P values below 0.05 were considered as significant. All statistical analyses were per-protocol analyses. All participants were requested to report any adverse effects. Written informed consent was obtained from the patients. The medical ethics committee of the Ebne Sina Research Institute affiliated with the ACECR approved the protocol (approval number and date: 67/22/978 and 16 Sep. 2010). Further, the trial was registered in the Iranian Registry of Clinical Trials with the number IRCT201112252288N5.

Results

Determination of total phenolics content

The total phenolics content of the extract as milligrams of gallic acid per gram of the extract was 20.3 ± 1.7 (mean ± SD).

Patients

No adverse effects were reported. The groups were matched in regard to demographic data (age, gender, duration of diabetes and body mass index) (Table 1). The baseline blood levels of all parameters were not significantly different between the two groups (P > 0.05) (Table 2).

The extract lowered the blood levels of total cholesterol and LDL significantly (P=0.002 and P=0.040 respectively) without any significant effects on the other blood parameter levels (P>0.05) compared with the placebo group at the endpoint (Table 2).

Table 1- The demographic data of the subjects who completed the trial. The data are given as mean ± SD

Parameter	Artichoke group	Placebo group
Age (years)	51.5 ± 9.3	50.6 ± 11.8
Gender	8 males, 30 females	10 males, 28 females
Duration of type 2 diabetes mellitus (years)	6.3 ± 3.1	8.4 ± 4.3
Weight	70.2 ± 9.7	68.7 ± 8.9

Table 2- The blood parameter levels before and after intervention. * = P<0.05 = significant (Mann-Whitney U test). A (artichoke); P (placebo); SD (standard deviation)

Parameter	Mean (SD) before	P value	Mean (SD) after	P value
Fasting glucose (mg/dL)	178 (25) A	>0.05	167 (30) A	>0.05
	166 (24) P		159 (32)	
2 h postprandial glucose	238. 2 (19.7) A	>0.05	223.4 (36.2)	>0.05
	252.2 (26.7) P		241.8 (25.6)	

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Table 2- Continued

Parameter	Mean (SD) before	P value	Mean (SD) after	P value
HbA1c (%)	7.2 (1.1) A 7.1 (1.4) P	>0.05	6.9 (1.0) A 7.6 (1.7) P	>0.05
Total cholesterol (mg/dL)	223 (20) A 233 (14) P	>0.05	208 (16) A 238 (12) P	*0.002
Triglycerides (mg/dL)	228.6 (65.7) A 195.0 (84.3) P	>0.05	187.4 (73.5) A 180.9 (94.1) P	>0.05
LDL (mg/dL)	126 (11) A 134 (18) P	>0.05	118 (16) A 140 (21) P	*0.040
HDL (mg/dL)	45.1 (12.3) A 45.7 (11.0) P	>0.05	42.9 (11.3) A 43.2 (9.3) P	>0.05
SGOT (U/L)	21.3 (6.3) A 20.3 (5.3) P	>0.05	19 (5.9) A 22.9 (16.5) P	>0.05
SGPT (U/L)	15.3 (6.9) A 15.6 (7.2) P	>0.05	12.1 (4.9) A 14.5 (7.1) P	>0.05
Creatinine (mg/dL)	0.9 (0.1) A 0.9 (0.1) P	>0.05	0.9 (0.1) A 0.9 (0.1) P	>0.05

Discussion

The fiber-free artichoke leaf extract did not improve glycemic control, but lowered the blood levels of total cholesterol and LDL without effects on the other parameter levels and hepatic, renal or other adverse effects in the hypercholesterolemic type 2 diabetic patients. The results suggest that dietary fibers may be involved in the anti-hyperglycemic effect of artichoke in the type 2 diabetic patients, but they do not seem to have a significant role in the anti-hypercholesterolemic effect of artichoke.

As far as is known after searching various databases, the bioactives and mechanisms involved in the anti-hyperglycemic effect of artichoke are not yet characterized and only one study concerning the bioactives and mechanism mediating the anti-hypercholesterolemic effect of artichoke has been conducted. Artichoke leaf aqueous extract and constituents cynaroside, luteolin and chlorogenic acid blocked cholesterol biosynthesis in primary rat hepatocytes by inhibition of hydroxymethylglutaryl-CoA-reductase activity [12]. Further, the only bioactives that were identified and quantified in the extract used in the current trial were total phenolics. The bioactives and mechanisms involved in the anti-hypercholesterolemic action of the artichoke extract were not examined in the present trial.

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Thus, in view of the results of this trial, further trials concerning the effects of artichoke in the treatment of patients with T2DM and/or various types of hyperlipidemias as well as more studies addressing the mechanisms of the bioactives involved in the anti-hypercholesterolemic and anti-hyperglycemic actions of artichoke seem necessary.

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