The effects of Anethum graveolens (dill) powder supplementation on clinical and metabolic status in patients with type 2 diabetes

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Abstract
Background: The objective of this study was to investigate the effects of anethum graveolens (dill) powder supplementation on glycemic control, lipid profile, some antioxidants and inflammatory markers, and gastrointestinal symptoms in type 2 diabetic patients. Material and methods: In this study, 42 patients with type 2 diabetes were randomly allocated to intervention and control groups and received either 3g/day dill powder or placebo (3 capsules/day, 1 g each). Fasting blood sugar (FBS), insulin, homeostatic model assessment of insulin resistance (HOMA-IR), lipid profile, hs-C-reactive protein (hs-CRP), total antioxidant capacity (TAC), malondialdehyde (MDA), and gastrointestinal symptoms were measured in all of the subjects at baseline and post-intervention. Results: The dill powder supplementation significantly decreased the mean serum levels of insulin, HOMA-IR, LDL-C, TC, and MDA in the intervention group in comparison with the baseline measurements (p < 0.05). Also, the mean serum levels of HDL and TAC were significantly increased in the intervention group in comparison with the baseline measurement (p < 0.05). Colonic motility disorder was the only gastrointestinal symptom whose frequency was significantly reduced by supplementation (P = 0.01). The mean changes of insulin, LDL-C, TC, and MDA were significantly lower in the intervention group than in the control group (p < 0.05). In addition, the mean changes in HDL were significantly higher in the intervention group than in the control group (p < 0.05). Conclusion: Dill powder supplementation can be effective in controlling the glycemic, lipid, stress oxidative, and gastrointestinal symptoms in type 2 diabetic patients. Keywords: Type 2 diabetes; Dill powder; Glycemic control; Lipid profile; Stress oxidative status

Introduction
Diabetes is a public health problem that affected 285 million adults in 2010. That number is expected to rise to 439 million—or 7.7% of all adults—by 2030 (1). In Iran, it has been estimated that 8% of the adult population has diabetes (2). Major characteristics of type 2 diabetes mellitus (T2DM) are obesity, impaired insulin action, insulin secretory dysfunction, and increased endogenous glucose output (3). Increased free fatty acid flux secondary to insulin resistance is associated with diabetic dyslipidemia, including high plasma triglyceride concentration and low HDL cholesterol concentration.
Inflammatory cytokines contribute to T2DM occurrence by affecting beta-cell function, which, in turn, promotes the long-term complications of diabetes by intensifying hyperglycemia (5). Increased glucose uptake by endothelial cells in hyperglycemic conditions also leads to the increased production of free radicals, which decreases antioxidant levels (6). It is commonly reported that patients with T2DM also encounter gastrointestinal complications, including gastroesophageal reflux disease (GERD), gastroparesis, enteropathy, nonalcoholic fatty liver disease (NAFLD), and glycogenic hepatopathy (7).

Anethum graveolens L (commonly referred to as dill), is a herb commonly used both as a remedy and as a spice (8). It grows in the Mediterranean region, Europe, central and southern Asia, and the southeastern region of Iran (9). *Anethum graveolens* (AG) leaves are a source of minerals, proteins, and fibres (10). AG oils are also a source of antioxidants and have antimicrobial and antispasmodic properties (11). In traditional herbal medicine, AG is used to treat gastrointestinal ailments such as indigestion and flatulence (12). AG has been established to have anticancer, antimicrobial, antigastric irritation, anti-inflammatory, and antioxidant properties (13). In diabetic models, the administration of different extractions of AG seed had antioxidant, hypolipidemic, and hypoglycemic effects (14).

Earlier studies have reported inconsistent findings regarding the protective effects of AG on lipid profile and insulin resistance in patients with metabolic syndrome (15, 16). Randomized clinical trials showed that AG reduced total cholesterol and low-density lipoprotein cholesterol (LDL-C) but did not change triglyceride and high-density lipoprotein cholesterol (HDL-C) in patients with T2DM (17). It has also been reported that AG could have beneficial effects on some inflammatory biomarkers (18) and controversial effects on glucose and insulin (18, 19). Given the inconclusive results related to glycemic, lipid and inflammatory profiles, it is not clear whether AG helps to increase antioxidants or improve gastrointestinal symptoms. Therefore, the present study was designed to examine the effects of AG powder on the serum levels of glycemic parameters, lipid profile, some antioxidants, inflammatory markers, and gastrointestinal symptoms in patients with type 2 diabetes.

**Materials And Methods**

**Study design and participants**
A single-centre randomized double-blind placebo-controlled study was conducted with 100 type 2 diabetes patients. The patients were recruited from the endocrinology and metabolism clinics of Golestan Hospital at Ahvaz Jundishapur University of Medical Science in Iran between 2017 and 2018 (Fig. 1).

Inclusion criteria: patient has DM; is aged 30-60 years; has gastrointestinal symptoms; has a body mass index (BMI) between 25 and 35 kg/m²; does not have systemic diseases, thyroid disease, or kidney disorder; is not pregnant or lactating; and is not taking any dietary supplements or antioxidants, immunosuppressants, or anti-inflammatory agents. Exclusion criteria: patient shows noticeable changes in the dose of medications and treatment of diabetes, refuses to continue participating in the study, or has less than 90% compliance with dill capsules.

Diagnosis of DM was done according to American Diabetes Association guidelines. Patients with FBS ≥ 126 mg/dl or (2-hour glucose) 2 hpp ≥ 200 mg/dl, or HbA1c ≥ 6.5% were diagnosed with diabetes mellitus (20).

Fifty-two patients did not qualify for this study due to not meeting inclusion criteria such as gastrointestinal symptoms and not accepting to participate. Forty-eight patients were randomly assigned to two groups of intervention (n = 24) or placebo (n = 24), for 8 weeks. Randomization was done using the computer-generated random numbers by a third person to reduce the bias. The third person were generated a random block in blocks of 4. The naming of Dill or placebo bottles were done according to random numbers. Odd or even numbers were allocated randomly to groups A or B. A multi-part questionnaire including demographic data (age and sex), anthropometric indices, dietary intake, medication, diabetes duration (in years), physical activity, and gastrointestinal symptoms was obtained from the subjects. During each visit, every patient was given dill supplement or placebo for 4 weeks and throughout these weeks, consumption of supplements or placebo by the patients was ensured through phone calls or text messages. The compliance of patients was checked by counting the remaining capsules. Patients were excluded from study if they had consumed less than 90% of the prescribed capsules. All participants were asked not to consume dill in their diet during the study. The protocol of this study was approved by the Ethics Committee of Ahvaz Jundishapur University of
Medical Sciences (Ethical Code: IR.AJUMS.REC.1396.623) and this study was registered in the Iranian Registry of Clinical Trials website (IRCT20120704010181N12) which is available at: http://irct.ir/user/trial/20288/view. Written informed consent was obtained from all participants.

**Supplement and placebo prescription**

After confirmation of the Anethum Graveolens (dill) herb by the botanist, dried leaves were milled to powder. Capsules containing 1 g of dill powder were provided by the Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences. In this study, starch was used as placebo. The intervention and control groups received either 3 capsules of 1 gr dill or placebo three times per day after each meal (breakfast, lunch and dinner) for 8 weeks. The placebo and dill capsules were matched with together in terms of size, taste, color and shape.

**Assessment of demographic data, anthropometric indices and food intakes**

Dietary intakes were investigated with a 24-h food recall for 3 days (2 weekdays and 1 weekend day), and dietary intake was analyzed by Nutritionist 4 software specified for Iranian foods. Anthropometric indices (weight, height, BMI) were measured by a trained researcher (nutritionist) at baseline and after the 8-week intervention. Weight (Seca, Germany) was measured while the patients wore light clothing and no shoes with 0.1-kg accuracy for weight. Height was measured using a stadiometer (Seca) with 0.5-cm accuracy without shoes. BMI was calculated (weight in kilogram divided by the square of the height in meter). Physical activity level was evaluated by the Persian form of the International Physical Activity Questionnaire (IPAQ) and presented in Met-Min/week. The participants were asked not to change their ordinary dietary intake and physical activity during the intervention.

**Assessment of gastrointestinal symptoms**

The assessment of gastrointestinal symptoms was done by questionnaire at the baseline and end of the study (21). This questionnaire was included gastrointestinal symptoms such as gastroesophageal reflux, esophageal motility disorders, dyspepsia, gastric motility disorders and colonic motility disorders.

The numbers 0, 1 and 2 indicate the severity of gastrointestinal symptoms. 0: the patient did not
have gastrointestinal symptoms, 1: patient had occasional gastrointestinal symptoms, and 2 ≤: the patient had permanently gastrointestinal problems.

**Biochemical assays**

Fasting blood samples (5 ml) were collected from all participants at the beginning and end of the study and were immediately centrifuged (3000×g, 10 min, 4°C). Blood samples were poured into anticoagulant tubes in order to extract serum samples and sent to the lab in cool boxes. All samples were stored at −70 °C until biochemical analyses. Serum glucose, TG, HDL and TC was measured by the standard enzymatic methods using Pars Azmoun kit (Tehran, Iran). Serum insulin was measured by human insulin enzyme-linked immunosorbent (ELISA) kit (monobind). Insulin resistance was estimated according to the Homeostasis Model Assessment (HOMA) calculated as: HOMA-IR = fasting concentrations of glucose (mg/dL) × fasting insulin (μU/mL) / 405 (22). Friedewald formula was used for calculation of LDL (23):

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\text{LDL-c (mg/dL) = TC (mg/dL) − HDL-c (mg/dL) − TG (mg/dL)/5 (VLDL), VLDL} = \text{TG (mg/dL)/5}
\]

Serum markers of oxidative stress such as total antioxidant capacity (TAC) and malondialdehyde (MDA) were measured by reliable spectrophotometric methods using Zell Bio GmbH kit (Germany). Serum levels of hc-CRP were measured by enzyme-linked immunosorbent assay (ELISA) kits (Diagnostics monobind).

**Outcomes**

In this study, LDL-C was considered as the primary outcome. Also, the secondary measurements outcomes were glycemic parameters, other factors of lipid profile, some antioxidant and inflammatory markers, and gastrointestinal symptoms.

**Statistical analysis**

The sample size (95% confidence interval and 80% power) was computed according to Mobasseri and et al study (17) and considering LDL-C as the main outcome. Sample size was 21 subjects for each group. 24 subjects were computed in each group with 10% withdrawal. All statistical analyses was performed using SPSS 25. All data were reported as mean ± standard deviations (SD) for quantitative variables or number (percentage) for qualitative variables. Normal distribution of data was checked
using Kolmogorove-Smirnov test. Paired sample t-test was also used to compare the results within groups post-intervention. Independent sample t-test was performed to compare the results between the two groups (placebo and intervention). Also, Independent T-test was used to identify differences between the two groups at the end of the study. The mean changes of variables was calculated using the mean differences of data before and after the study. Analysis of covariance (ANCOVA) was used to identify any differences between two groups at the end of the study, adjusting for baseline values and covariates. Also, Chi square test was used for statistical analysis of qualitative variables. P-value of less than 0.05 was considered statistically significant in all analyses.

Results

Baseline characteristics of the subjects, anthropometric parameters and dietary intake

42 diabetic patients (intervention group n = 21; control group = 21) for 8 weeks completed the study. The mean age of patients in the intervention and control groups was 50.66 ± 8.22 and 50.42 ± 8.61 years, respectively. No significant differences (P ≥ 0.05) were observed in demographic and anthropometric characteristics, duration of diabetes, physical activity and medications between the two groups at baseline (Table 1). No significant differences were also observed between the two groups for dietary intake including energy, macronutrients and micronutrients such as antioxidant vitamins C and E at baseline and after the intervention (P ≥ 0.05) (Table 2).

Glycemic control

The results of this study showed that no significant differences were observed in FBS, insulin and HOMA-IR between 2 groups at baseline (P ≥ 0.05). It was demonstrated that 8 weeks consumption of dill powder significantly decreased the mean serum levels of insulin and HOMA-IR in the intervention group in compare with baseline (13.27± 3.8 vs 10.54 ± 4.51 µU/ml, respectively; P = 0.004), HOMA-IR (4.88 ± 2.37 vs 3.86 ± 2.32, respectively; P = 0.039). Furthermore, the mean changes of insulin was significantly lower in the intervention group in compare with control group after the intervention (-2.7 ± 3.83 vs 0.50 ± 4.36, respectively; P = 0.015). Analysis of covariance (ANCOVA) showed that after the adjusting of confounding factors (age, duration of disease, changes of body mass index, dietary intake of energy, macronutrients, Vitamin A, C, and E, and physical activity), the mean changes of
insulin were not significantly (P = 0.05) lower in the intervention group in comparison with control group after the intervention (Table 3).

**Lipid profile**

At baseline, there were no significant differences in the mean serum levels of TG, TC, LDL-C and HDL between two groups (P > 0.05). The dill powder supplementation significantly increased the mean serum levels of HDL in the intervention group in comparison with baseline (44.80 ± 9.89 to 41.85 ± 11.68 mg/dl, respectively; P = 0.007). Also, the mean changes of serum levels of HDL were significantly higher in the intervention group compared to the control group (2.59 ± 4.51 vs -1.38 ± 4.60 mg/dl, respectively; P = 0.004). Even after the adjusting of confounding factors, there was a significant difference in mean change of HDL-C between two groups (P = 0.04). In the intervention group, it was shown that the mean serum levels of LDL-C and TC significantly decreased post-intervention (81.00 ± 34.79 to 71.23 ± 26.63 mg/dl, respectively; p = 0.029), TC (160.28 ± 38.26 to 149.23 ± 26.7 mg/dl, respectively; p = 0.03). Furthermore, the mean changes of serum levels of LDL-C were significantly lower in the intervention group compared to the control group (-9.76 ± 19.08 vs 3.09 ± 14.07 mg/dl, respectively; P = 0.017). After the adjusting of confounding factors, there was a significant difference in mean change of LDL-C and TC between two groups (P = 0.04 and P = 0.033, respectively). However, no significant changes were observed in the mean serum levels of TG after the intervention (P ≥ 0.05) (Table 3).

**Antioxidant and inflammatory markers**

According to the analysis, there were no significant differences in the mean serum levels of hs-CRP, MDA and TAC between two intervention and control groups at the baseline (P ≥ 0.05). The results of present study showed that in intervention group the mean of MDA was reduced significantly post-intervention in comparison with baseline (3.34 ± 2.05 to 2.22 ± 1.57 μM, respectively; P = 0.034). At the end of study, there was a significant difference in the mean changes of MDA between intervention and control groups without and with the adjusting of confounding factors (-1.11 ± 2.24 vs 0.33 ± 1.62 μM, respectively; P = 0.021 vs P = 0.013, respectively). Within group comparison in the intervention group showed that the mean serum levels of TAC significantly increased after 8 weeks of
supplementation (0.19 ± 0.05 to 0.25 ± 0.09 mM, respectively; p=0.025). In addition, after the supplementation, the mean serum levels of TAC were significantly higher in the intervention group in comparison with the control group (0.25 ± 0.09 vs 0.16 ± 0.06 mg/dl, respectively; P = 0.001). This result for TAC was also observed after the adjusting of confounding factors (P = 0.004). No significant difference was observed for hs-CRP within and between the two groups (P ≥ 0.05) (Table 4).

**Gastrointestinal symptoms**

Based on the results presented in Table 5, supplementation with dill failed to reduce the frequency of gastrointestinal symptoms such as gastroesophageal reflux, esophageal motility, dyspepsia, and gastric motility disorders in comparison with the baseline measurements (P ≥ 0.05). Amongst all the symptoms, only colonic motility disorders had their frequency significantly reduced by supplementation (P = 0.01), and this decrease was more notable in patients with severe gastrointestinal problems. In the control group, meanwhile, there was no significant reduction in the frequency of gastrointestinal symptoms (P ≥ 0.05).

**Safety, adverse effects and monitoring data**

A Data Monitoring Committee (DMC) was supervised this study to detect any possible side effects and report to the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences. However, no significant side effects from dill administration were reported in this study.

**Discussion**

This study revealed that 8 weeks of supplementation with 3 g/day AG reduced serum insulin and HOMA-IR. Moreover, AG might significantly reduce the serum levels of LDL and TC and enhance HDL when compared to a placebo condition. Patients in the intervention group had low MDA and TAC; however, no significant changes were observed for the serum levels of hs-CRP. In terms of gastrointestinal symptoms, only colonic motility disorders decreased.

These findings are in line with those of several interventional studies confirming the benefits of AG in improving T2DM and metabolic syndrome (14, 16). The significant reduction in HOMA-IR and serum levels of insulin indicates that AG has a role to play in reducing insulin resistance. Similar beneficial effects of AG on glycemic control have been reported previously. Supplementation of T2DM patients
with 3.3 g/day of powder of Anethum for 8 weeks could significantly reduce levels of insulin (17). After 6 weeks of supplementation with 1.5g/day of dill powder tablets, serum levels of FBS were significantly reduced in patients with T2DM (19).

Although Payahoo et al. (18) found a significant decrease in serum levels of insulin, no significant effect was observed for HOMA-IR, which could be due to the reduced levels of FBS in diabetic patients. High antioxidant content (i.e., vitamin C, polyphenols, and carotenoids) in AG neutralizes reactive oxygen species and thus plays a role in repairing beta-cell function and insulin secretion (24, 25).

In this study, serum concentrations of LDL-C and TC decreased, while HDL-C increased significantly at the end of the study. No significant change was seen for serum levels of TG. In agreement with our study, Rashidlamir et al. (26) showed that aerobic training with the use of 2.7 g/day of AG resulted in increased HDL and a decreased LDL-to-HDL ratio in diabetic women compared with the control group; findings for TC, meanwhile, was not statistically significant. In contrast, supplementation with 650 mg of anethum tablets twice daily increased the serum levels of TG in patients with hyperlipidemia, but no significant changes were seen in TC or LDL (15).

The treatment of hyperlipidemic patients with 1 g/day of AG powder for 4 weeks resulted in a significant reduction in the levels of TC, TG, LDL and VLDL when compared to patients treated with 20 mg/day of lovastatin tablets. However, no significant change was observed in the serum levels of HDL(27).

The exact mechanism of the lipid-lowering effects of AG is not yet determined. However, it may relate to the decreased absorption of cholesterol by binding to bile acids, the inhibition of cholesterol and fatty acid synthesis through the suppression of acetyl-CoA carboxylase and HMG-COA reductase activity and the stimulation of cholesterol clearance by increasing LDL receptors (28-30).

In this study, patients who received 3 g/day of AG had lower levels of MDA and higher levels of TAC than patients in the control group, both in crude and adjusted models. MDA is a product of lipid peroxidation and is recognised as an atherogenic agent. Patients with elevated levels of MDA are more susceptible to atherosclerosis, diabetes, and other metabolic disorders (31).
Findings from animal studies showed that the administration of different fractions of AG to animals on a high-fat diet decreased their MDA levels and increased the activities of antioxidant enzymes, including superoxide dismutase (SOD) and catalase. It also increases the levels of glutathione (GSH), thus playing a key role in scavenging ROS (32). Hamsters treated with AG extracts or tablets exhibited a significant increase in TAC levels when compared to those on a high-cholesterol diet (33). AG is composed of a variety of antioxidants, such as flavonoids—capable of scavenging free radicals (34). The enhanced levels of antioxidant activity in response to AG might be due to the content of polyphenols and flavonoids. It’s possible that normal levels of antioxidants protect individuals against several chronic diseases (35).

We observed a non-significant decrease in serum levels of hs-CRP after supplementation with AG. The fact that an increase in body weight is an indicator of inflammation (36) could be why a non-significant reduction in serum levels of hs-CRP was observed in our study. The anti-inflammatory effects of different forms of AG have been shown in several animal studies (37-39). Payahoo et al. (18) found a significant decrease in the serum levels of inflammatory biomarkers—including hs-CRP, IL-6, and TNF-α—after 8 weeks of supplementation with 3.3 grams of dill powder.

In terms of gastrointestinal symptoms, we observed a significant decrease in colonic motility disorders only. It is reported that the most prevalent symptoms among diabetic patients are colonic motility disorders, which increase with age (21). The prevalence of gastrointestinal symptoms is positively associated with the duration of diabetes (21, 40). Patients included in the current study had a mean age of 50 years and a mean disease duration of 8 years—both of which are relatively high. This could be a reason for the observed findings in this regard. Earlier animal models show that AG extract is a potent relaxant of contractions in rat ileum and has antisecretory and anti-ulcer capabilities as it relates to HCl- and ethanol-induced stomach lesions (41, 42).

To the best of our knowledge, this is the first human study investigating the effects of AG on gastrointestinal symptoms. The major strength of this study was its design as a well-controlled double-blind clinical trial that controlled for several main confounding factors in different models. However, there are some limitations to our study. First, this is a single-dose trial, thus preventing any
dose-effect associations. It remains unclear whether larger or smaller doses could introduce a stronger clinical effect. Second, the narrow range of inclusion criteria led to unrepresentative samples, therefore limiting the generalizability of the study results to all diabetic patients. Third, only the data of subjects who completed the study were analyzed; the data of those who were excluded were not measured.

Conclusion
In conclusion, the present study suggests beneficial effects of AG in insulin resistance, LDL and HDL cholesterol, antioxidant levels, and some gastrointestinal symptoms compared with placebo during 8 weeks of supplementation. Further studies are needed to determine molecular levels and clarify its role in the treatment of diabetes complications.

Abbreviations
Fasting blood sugar (FBS), gastro-esophageal reflux disease (GERD), high-density lipoprotein (HDL), homeostatic model assessment of insulin resistance (HOMA-IR), hs-C-reactive protein (hs-CRP), low-density lipoprotein (LDL), malondialdehyde (MDA), non-alcoholic fatty liver disease (NAFLD), total antioxidant capacity (TAC), total cholesterol (TC), triglyceride (TG).

Declarations
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Author contribution
Amoochi G, Haidari F concepted the idea and designed the study. Amoochi G and Zakerkish M collected the data. Haidari F and Ahmadi Angali K analyzed and interpreted the results. Amoochi G, Haidari F and Borazjani F drafted the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

The results will not be available before publishing.

**Ethics approval and consent to participate**

The protocol was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (Ethical Code: IR.AJUMS.REC.1396.623) that is in accordance with the Declaration of Helsinki.

Each participant will sign an informed consent form.

**Consent for publication**

Not applicable

**Competing interests**

The authors declare that they have no competing interests.

**References**

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes research and clinical practice. 2010 Jan;87(1):4-14. PubMed PMID: 19896746. Epub 2009/11/10. eng.

2. Esteghamati A, Gouya MM, Abbasi M, Delavari A, Alikhani S, Alaedini F, et al. Prevalence of diabetes and impaired fasting glucose in the adult population of Iran: National Survey of Risk Factors for Non-Communicable Diseases of Iran. Diabetes care. 2008 Jan;31(1):96-8. PubMed PMID: 17921357. Epub 2007/10/09. eng.

3. Rosal MC, Borg A, Bodenlos JS, Tellez T, Ockene IS. Awareness of diabetes risk factors and prevention strategies among a sample of low-income Latinos with no known diagnosis of diabetes. The Diabetes educator. 2011 Jan-Feb;37(1):47-55. PubMed PMID: 21220363. Epub 2011/01/12. eng.

4. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. Nature clinical practice Endocrinology & metabolism. 2009 Mar;5(3):150-9. PubMed PMID: 19229235. Epub 2009/02/21. eng.

5. Lontchi-Yimagou E, Sobngwi E, Matsha TE, Kengne AP. Diabetes mellitus and
inflammation. Current diabetes reports. 2013 Jun;13(3):435-44. PubMed PMID: 23494755. Epub 2013/03/16. eng.

6. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. The Journal of clinical investigation. 2005;115(5):1111-9. PubMed PMID: 15864338. eng.

7. Krishnan B, Babu S, Walker J, Walker AB, Pappachan JM. Gastrointestinal complications of diabetes mellitus. World journal of diabetes. 2013;4(3):51-63. PubMed PMID: 23772273. Epub 2013/06/15. eng.

8. Saleh-E-In MM, Sultana N, Rahim MM, Ahsan MA, Bhuiyan MNH, Hossain MN, et al. Chemical composition and pharmacological significance of Anethum Sowa L. Root. BMC complementary and alternative medicine. 2017;17(1):127-. PubMed PMID: 28231789. eng.

9. V. M. Dictionary of Iranian plant names. Tehran, Iran. Farhang Moaser Publications. 1995:44.

10. Rekha MN YA, Dharmesh Sh, Chauhan AS, Ramteke RS. Evaluation of Antioxidant properties of dry soup mix extracts containing Dill (Anethum sowa L.) leaf. Food Bioprocess Technol 2010;3:441-449.

11. Singh G, Maurya S, De Lampasona M, Catalan C. Chemical constituents, antimicrobial investigations, and antioxidative potentials of Anethum graveolens L. essential oil and acetone extract: Part 52. 2005;70(4):M208-M15.

12. NJ: HDM. PDR for herbal medicines. Thomson. 2004:650-1. English.

13. Oshaghi EA, Khodadadi I, Tavilani H, Goodarzi MT. Effect of dill tablet (Anethum graveolens L) on antioxidant status and biochemical factors on carbon tetrachloride-induced liver damage on rat. International Journal of Applied and Basic Medical Research. 2016;6(2):111.

14. Goodarzi MT, Khodadadi I, Tavilani H, Abbasi Oshaghi E. The Role of Anethum
graveolens L. (Dill) in the Management of Diabetes. Journal of tropical medicine. 2016;2016:1098916. PubMed PMID: 27829842. Pubmed Central PMCID: PMC5088306. Epub 2016/11/11. eng.

15. Kojuri J, Vosoughi AR, Akrami M. Effects of anethum graveolens and garlic on lipid profile in hyperlipidemic patients. Lipids in Health and Disease. 2007;6(1):5.

16. Mansouri M, Nayebi N, Hasani-Ranjbar S, Taheri E, Larijani B. The effect of 12 weeks Anethum graveolens (dill) on metabolic markers in patients with metabolic syndrome; a randomized double blind controlled trial. DARU Journal of Pharmaceutical Sciences. 2012;20(1):47.

17. Mobasseri M, payahoo I, Ostadrahimi A, Khaje bishak Y, Asghari Jafarabadi M, Mahluji S. Anethum graveolens Supplementation Improves Insulin Sensitivity and Lipid Abnormality in Type 2 Diabetic Patients. 2014 2014/9/30 %J Pharm Sci;20(2):40-5.

18. Payahoo L K-BY, Mobasseri M, Ostadrahimi A, Asghari-Jafarabadi M. The Effects of Anethum Graveolens L Supplementation on the Insulin Resistance and Inflammatory Biomarkers in Patients with Type 2 Diabetes. JIMS 2015; 32(320): 2473-83.

19. Sargolzari MS MA, Shahdadi H, Masinaei Nezhad N, Poodineh Moghadam M. The effect of dill tablet on the level of fasting blood sugar in patients with type II diabetes. J Diabetes Nurs 2017;5(2):86-94.

20. Ta S. Diagnosis and classification of diabetes mellitus. Diabetes care. 2014;37:S81.

21. Shahbazian Hb, Hashemi SJ, Arghideh M, Fardad F, Latifi SM. Prevalence of Gastrointestinal Symptoms in Type 2 Diabetic Patients and its Association with Glycemic Control and Duration of Diabetes %J Iranian Journal of Endocrinology and Metabolism. 2012;13(5):459-66. eng.

22. Fujii H, Imajo K, Yoneda M, Nakahara T, Hyogo H, Takahashi H, et al. HOMA-IR: An independent predictor of advanced liver fibrosis in nondiabetic non-alcoholic fatty
liver disease. Journal of gastroenterology and hepatology. 2019.

23. Moravej Aleali A, Amani R, Shahbazian H, Namjooyan F, Latifi SM, Cheraghian B. The effect of hydroalcoholic Saffron (Crocus sativus L.) extract on fasting plasma glucose, HbA1c, lipid profile, liver, and renal function tests in patients with type 2 diabetes mellitus: A randomized double-blind clinical trial. Phytotherapy Research. 2019.

24. Agte VV, Tarwadi KV, Mengale S, Chiplonkar SA. Potential of Traditionally Cooked Green Leafy Vegetables as Natural Sources for Supplementation of Eight Micronutrients in Vegetarian Diets. Journal of Food Composition and Analysis. 2000 2000/12/01/;13(6):885-91.

25. Madani H, Ahmady Mahmoodabady N, Vahdati A. Effects of hydroalchoholic extract of Anethum graveolens (Dill) on plasma glucose an lipid levels in diabetes induced rats. Iranian Journal of Diabetes and Metabolism. 2005 Sep 15;5(2):109-16.

26. Rashidlamir A, Gholamian S, Javaheri AH, Dastani M. The effect of 4-weeks aerobic training according with the usage of Anethum graveolens on blood sugar and lipoproteins profile of diabetic women. Annals of Biological Research. 2012 Aug 12;3(9):4313-9.

27. Sahib AS, Mohammad IH, AlGareeb A. Effects of Anethum graveolens leave powder on lipid profile in hyperlipidemic patients. Spatula DD. 2012;2(3):153-8.

28. Yazdanparast R, Bahramikia S. Evaluation of the effect of Anethum graveolens L. crude extracts on serum lipids and lipoproteins profiles in hypercholesterolaemic rats. DARU Journal of Pharmaceutical Sciences. 2008;16(2):88-94.

29. Piri M, Shahin M, Oryan S. The effects of Anethum on plasma lipid and lipoprotein in normal and diabetic rats fed high fat diets. Journal of Shahrekord University of Medical Sciences. 2010;11(4):15-25. eng.

30. Haghighi B, Kharazizadeh M, Attar M. Possible Involvement of Hepatic Phosphatidate
Phosphohydrolase in the Mechanisms of Actions of Certain Antilipemic Drugs in Rats

31. Bakhtiari A, Hajian-Tilaki K, Omidvar S, Nasiri Amiri F. Association of lipid peroxidation and antioxidant status with metabolic syndrome in Iranian healthy elderly women. Biomedical reports. 2017;7(4):331-6. PubMed PMID: 28928971. Epub 2017/08/09. eng.

32. Bahramikia S, Yazdanparast R. Efficacy of different fractions of Anethum graveolens leaves on serum lipoproteins and serum and liver oxidative status in experimentally induced hypercholesterolaemic rat models. The American journal of Chinese medicine. 2009;37(04):685-99.

33. Abbasi-Oshaghi E, Khodadadi I, Tavilani H, Mirzaei F, Goodarzi MT. Dill-normalized liver lipid accumulation, oxidative stress, and low-density lipoprotein receptor levels in high cholesterol fed hamsters. ARYA atherosclerosis. 2018;14(5):218-24. PubMed PMID: 30783412. eng.

34. Mateos R, Lecumberri E, Ramos S, Goya L, Bravo L. Determination of malondialdehyde (MDA) by high-performance liquid chromatography in serum and liver as a biomarker for oxidative stress. Application to a rat model for hypercholesterolemia and evaluation of the effect of diets rich in phenolic antioxidants from fruits. Journal of chromatography B, Analytical technologies in the biomedical and life sciences. 2005 Nov 15;827(1):76-82. PubMed PMID: 16009604. Epub 2005/07/13. eng.

35. Kaplan M, Aviram M. Oxidized low density lipoprotein: atherogenic and proinflammatory characteristics during macrophage foam cell formation. An inhibitory role for nutritional antioxidants and serum paraoxonase. Clinical chemistry and laboratory medicine. 1999 Aug;37(8):777-87. PubMed PMID: 10536926. Epub
36. Adebayo AH, Abolaji, A. O., Opata, T. K., and Adegbenro, I. K. . Effects of ethanolic leaf extract of Chrysophyllum albidum G. on biochemical and haematological parameters of albino Wistar rats. African Journal of Biotechnology. 2010;9:2145-50.

37. Valady A NS, Abbasi N. Anti-inflammatory and analgesic effects of hydroalcoholic extract from the seed of Anethum graveolens L. J Med Plants 2010; 9: 130-124.

38. Naseri M MF, Khodadoost M. The study of antiinflammatory activity of oil-based dill (Anethum graveolens L.) extract used topically in formalin-induced inflammation male rat paw. Iranian Journal of Pharmaceutical Research 2012; 11 (4): 1169-1174.

39. and TPA-induced mouse ear edema. Zhonghua Yaoxue Zazhi 1995; 47:421-430.

40. Fujishiro M, Kushiyama A, Yamazaki H, Kaneko S, Koketsu Y, Yamamotoya T, et al. Gastrointestinal symptom prevalence depends on disease duration and gastrointestinal region in type 2 diabetes mellitus. World journal of gastroenterology. 2017 Sep 28;23(36):6694-704. PubMed PMID: 29085214. Pubmed Central PMCID: PMC5643290. Epub 2017/11/01. eng.

41. Naseri MG, Heidari A. Antispasmodic effect of Anethum graveolens fruit extract on rat ileum. Int J Pharmacol. 2007;3:260-4.

42. Hosseinzadeh H, Karimi GR, Ameri M. Effects of Anethum graveolens L. seed extracts on experimental gastric irritation models in mice. BMC Pharmacol. 2002;2:21-. PubMed PMID: 12493079. eng.

Tables
Table 1 Demographic and anthropometric characteristics of participants at the baseline and at the end of the study.
| Variable                  | Dill powder (n=21) | Placebo (n=21) | P-value* |
|--------------------------|--------------------|----------------|----------|
| Duration of disease (year) | 8.1 ± 5.49        | 8.57 ± 6.72    | 0.714    |
| Weight (kg)              |                    |                |          |
| Baseline                 | 78.22 ± 11.08      | 77.29 ± 8.42   | 0.761    |
| End                      | 78.08 ± 11.07      | 77.30 ± 8.25   | 0.796    |
| P-value**                | 0.602              | 0.987          |          |
| BMI                      |                    |                |          |
| Baseline                 | 29.42 ± 3.24       | 28.95 ± 1.94   | 0.753    |
| End                      | 29.37 ± 3.29       | 28.95 ± 1.90   | 0.725    |
| P-value**                | 0.627              | 0.904          |          |
| Physical activity (MET-min/week) |          |                |          |
| Baseline                 | 1314.33 ± 1036.19  | 1428.66 ± 1053.76 | 0.725   |
| End                      | 1254.33 ± 960.52   | 1495.66 ± 953.10 | 0.419   |
| P-value**                | 0.626              | 0.451          |          |

Values are expressed as means ± SD. *P < 0.05 was considered as significant using Mann–Whitney U (for duration of disease and BMI) and Independent T-test (for other variables) between the two groups at baseline and after the intervention. **P < 0.05 was considered as significant using Wilcoxon Signed Ranks Test (for BMI) and Paired T-test (for other variables). ***P < 0.05 was considered as significant using chi-square test.

Table 2. Mean ± SD of energy, macronutrients and micronutrients intake at baseline and post-intervention
|                        | Dill powder (n=21) | Placebo (n=21) | P-Value $^1$ | P value $^2$ |
|------------------------|--------------------|----------------|--------------|--------------|
|                        | 1881 ± 161         | 1796 ± 167     | 0.100        | 0.113        |
|                        | 1861 ± 176         | 1811 ± 163     | 0.339        | 0.257        |
|                        | 0.412              | 0.593          |              |              |
|                        | 249.61 ± 23.19     | 240.40 ± 20.46 | 0.180        | 0.146        |
|                        | 246.51 ± 23.61     | 238.23 ± 14.28 | 0.179        | 0.113        |
|                        | 0.270              | 0.536          |              |              |
|                        | 75.41 ± 6.15       | 73.43 ± 6.09   | 0.303        | 0.240        |
|                        | 74.61 ± 5.72       | 72.04 ± 6.65   | 0.187        | 0.145        |
|                        | 0.210              | 0.135          |              |              |
|                        | 61.53 ± 5.37       | 59.66 ± 4.74   | 0.241        | 0.196        |
|                        | 60.26 ± 6.24       | 61.93 ± 4.32   | 0.169        | 0.098        |
|                        | 0.147              | 0.113          |              |              |
|                        | 377.38 ± 103.76    | 321.32 ± 85.71 | 0.064        | 0.034*       |
|                        | 368.66 ± 115.02    | 352.93 ± 88.03 | 0.622        | 0.693        |
|                        | 0.779              | 0.159          |              |              |
|                        | 87.03 ± 27.87      | 91.36 ± 29.58  | 0.628        | 0.744        |
|                        | 91.44 ± 30.92      | 96.85 ± 32.20  | 0.582        | 0.633        |
|                        | 0.592              | 0.541          |              |              |
|                        | 2.03 ± 0.72        | 2.38 ± 0.85    | 0.166        | 0.205        |
|                        | 1.85 ± 0.52        | 2.12 ± 0.63    | 0.146        | 0.136        |
|                        | 0.171              | 0.095          |              |              |

Values are expressed as means ± SD. $P <0.05$ was considered as significant.

**P-Value**: Between group comparison of variables at baseline and after intervention resulted from
Independent T-test (for all variables).

**P-Value2**: Between group comparison of variables at baseline and after intervention resulted from Analysis of Covariance (Ancova) in the adjusted models (adjusted for age, duration of disease, and body mass index).

**P-Value3**: Within group comparison of variables resulted from paired sample t test (for all variables)

Table 3. Serum levels of glycemic parameters and lipid profile at baseline and post-intervention.

|                        | Placebo (n=21) | Dill powder (n=21) | P-Value1 | P-Value2 | P-Value3 | P-Value4 |
|------------------------|----------------|--------------------|----------|----------|----------|----------|
| **Glucose**            | 148.61 ± 56.56| 145.76 ± 50.81     | 0.864    | 0.883    |          |          |
| **Insulin**            | 154.23 ± 36.72| 141.14 ± 40.37     | 0.278    | 0.623    |          |          |
| **HbA1c**              | 0.671          | 0.668              |          |          |          | 0.17     | 0.752    |
| **HDL**                | 5.61 ± 59.76   | -4.61 ± 48.56      |          |          |          |          |
| **LDL**                | 11.61 ± 4.91   | 13.273 ± 8        | 0.230    | 0.474    |          |          |
| **Total cholesterol**  | 12.12 ± 4.23   | 10.544 ± 5.1      | 0.250    | 0.796    |          |          |
| **Triglycerides**      | 0.604          | 0.004*             |          |          | 0.015*   | 0.05     |
| **Cholesterol**        | 0.50 ± 4.36    | -2.7 ± 3.83       |          |          |          |          |
| **Total cholesterol**  | 4.37 ± 3.01    | 4.88 ± 2.37       | 0.544    | 0.762    |          |          |
| **Triglycerides**      | 4.60 ± 2.02    | 3.86 ± 2.32       | 0.276    | 0.848    |          |          |
| **Cholesterol**        | 0.698          | 0.039*             |          |          |          |          |
| **Blood glucose**      | 0.23 ± 2.68    | -1.02 ± 2.12      |          |          | 0.101    | 0.447    |
| **HDL**                | 194.66 ± 75.58 | 196.52 ± 60.16    | 0.930    | 0.626    |          |          |
| **LDL**                | 190.19 ± 79.93 | 172.38 ± 69.86    | 0.447    | 0.664    |          |          |
| **Total cholesterol**  | 0.811          | 0.055              |          |          |          |          |
| **Triglycerides**      | -4.47 ± 84.69  | -24.14 ± 54.29    |          |          | 0.376    | 0.343    |
| **Cholesterol**        | 154.42 ± 32.42 | 160.28 ± 38.26    | 0.596    | 0.492    |          |          |
| **Blood glucose**      | 156.8 ± 32.25  | 149.23 ± 26.7     | 0.412    | 0.881    |          |          |
|                |       |       |       |       |
|----------------|-------|-------|-------|-------|
|                | 0.03* | 0.654 | 0.064 | 0.033*|
|                | -11.04 ± 21.7 | 2.38 ± 23.95 |       |       |
|                | 81.00 34.79 ± | 71.71 23.55 ± | 0.318 | 0.516 |
|                | 71.23 26.63 ± | 74.80 22.80 ± | 0.643 | 0.357 |
|                | 0.029* | 0.325 |       |       |
|                | -9.76 ± 19.08 | 3.09 ± 14.07 |       | 0.017* |
|                | 41.85 11.68 ± | 43.14 8.05 ± | 0.680 | 0.939 |
|                | 44.80 9.89 ± | 41.76 6.33 ± | 0.243 | 0.343 |
|                | 0.007* | 0.185 |       |       |
|                | 2.59 ± 4.51 | -1.38 ± 4.60 |       | 0.004* |

Values are expressed as means ± SD. *Statistically significant. **P-Value1**: Between-group comparison of variables at baseline and after intervention, resulted from Independent T-test (for all variables).  
**P-Value2**: Between-group comparison of variables at baseline and after intervention, resulted from Analysis of Covariance (Ancova) in the adjusted models (adjusted for age, duration of disease, dietary intake of energy, macronutrients, antioxidant vitamins such as vitamins A, C, and E, physical activity, and BMI).  
**P-Value3**: Between group comparisons mean Changes of variables resulted from Mann–Whitney U (for FBS) and Independent T-test (for other variables).  
**P-Value4**: Between group comparisons mean changes of variables resulted from Analysis of Covariance (Ancova) (adjusted for age, duration of disease, changes of body mass index, dietary intake of energy, macronutrients, Vitamin A, C, and E, and physical activity).  
**P-Value5**: Within-group comparison of variables, resulted from Paired T-test (for all variables).  
Abbreviations; Fasting blood sugar (FBS), homeostatic model assessment of insulin resistance (HOMA-IR), triglyceride (TG), total cholesterol (S), high-density (HDL) and low-density lipoprotein (LDL) cholesterol.

Table 4. The effects of dill supplementation on serum levels of antioxidant and inflammatory markers at baseline and post-intervention
|                    | Dill powder (n=21) | Placebo (n=21) | P-Value 1 | P-Value 2 | P-Value 3 | P-Value 4 |
|--------------------|--------------------|----------------|-----------|-----------|-----------|-----------|
| Placebo            | 3.72 ± 2.09        | 3.342 ± 2.05   | 0.554     | 0.054*    | 0.335     | 0.137     |
| Dill powder        | 0.17 ± 0.03        | 0.19 ± 0.05    | 0.103     | 0.001*    | 0.103     | 0.056     |
| Placebo            | 0.793              | 0.25 ± 0.09    | 0.001*    | 0.004*    | 0.339     | 0.145     |
| Dill powder        | -0.004 ± 0.7       | -0.058 ± 0.11  | 0.506     | 0.388     | 0.143     | 0.649     |
| Placebo            | 4.29 ± 0.70        | 4.13 ± 0.84    | 0.506     | 0.388     | 0.143     | 0.649     |
| Dill powder        | 4.32 ± 0.93        | 3.87 ± 0.89    | 0.122     | 0.143     | 0.649     | 0.649     |
| Placebo            | 0.872              | 0.283          | 0.332     | 0.332     | 0.649     | 0.649     |
| Dill powder        | 0.2 ± 0.8          | -0.25 ± 1.06   | 0.000*    | 0.000*    | 0.000*    | 0.000*    |

*Statistically significant. Values are expressed as means ± SD. P <0.05 was considered as significant.

P-Value1: Between-group comparison of variables at baseline and after intervention, resulted from independent sample t-test (for all variables).
P-Value2: Between-group comparison of variables at baseline and after intervention, resulted from analysis of covariance in the adjusted models (adjusted for age, duration of disease, dietary intake of energy, macronutrients, antioxidant, vitamins such as vitamins A, C, and E, physical activity, and BMI).
P-Value3: Between group comparisons mean changes of variables resulted from Mann-Whitney U (for TAC) and Independent T-test (for other variables).
P-Value4: Between group comparisons mean changes of variables resulted from Analysis of Covariance (Ancova) (adjusted for age, duration of disease, changes of body mass index, dietary intake of energy, macronutrients, Vitamin A, C, E, and physical activity).
P-Value5: Within-group comparison of variables, resulted from Paired T-test (for all variables).

Abbreviations; Hs-C-reactive protein (hs-CRP), total antioxidant capacity (TAC) and malondialdehyde (MDA)

Table5. The effects of dill powder supplementation on gastrointestinal symptoms at baseline and post-intervention
### Scores

| Gastrointestinal symptoms | Dill powder (n = 21) | Placebo (n= 21) |
|---------------------------|----------------------|-----------------|
|                           | 0                    | 1               | 2 ≤            | 0                | 1                |
| Gastroesophageal reflux   |                      |                 |                |                  |                  |
| Baseline                  | 5 (23.8%)            | 7 (33.3%)       | 9 (42.9%)      | 7 (33.3%)        | 5 (23.8%)        |
| End                       | 6 (28.6%)            | 9 (42.9%)       | 6 (28.6%)      | 7 (33.3%)        | 5 (23.8%)        |
| P-value                   | 0.135                |                 |                | 1.00             |
| Esophageal motility disorders |                  |                 |                |                  |                  |
| Baseline                  | 18 (85.7%)           | 1 (4.8%)        | 2 (9.5%)       | 16 (76.2%)       | 3 (14.3%)        |
| End                       | 16 (76.2%)           | 3 (14.3%)       | 2 (9.5%)       | 16 (76.2%)       | 3 (14.3%)        |
| P-value                   | 0.317                |                 |                | 1.00             |
| Dyspepsia                 |                      |                 |                |                  |                  |
| Baseline                  | 15 (71.4%)           | 2 (9.5%)        | 4 (19%)        | 14 (66.7%)       | 3 (14.3%)        |
| End                       | 12 (57.1%)           | 7 (33.3%)       | 2 (9.5%)       | 13 (61.9%)       | 5 (23.8%)        |
| P-value                   | 0.198                |                 |                | 0.368            |
| Gastric motility disorders |                      |                 |                |                  |                  |
| Baseline                  | 12 (57.1%)           | 1 (4.8%)        | 8 (38.1%)      | 11 (52.4%)       | 2 (9.5%)         |
| End                       | 10 (71.4%)           | 3 (14.3%)       | 3 (14.3%)      | 11 (52.4%)       | 2 (9.5%)         |
| P-value                   | 0.112                |                 |                | 1.00             |
| Colonic motility disorders |                      |                 |                |                  |                  |
| Baseline                  | 7 (33.3%)            | 3 (14.3%)       | 11 (52.4%)     | 6 (28.6%)        | 5 (23.8%)        |
| End                       | 10 (47.6%)           | 10 (47.6%)      | 1 (4.8%)       | 7 (33.3%)        | 4 (19%)          |
| P-value                   | 0.010*               |                 |                | 0.317            |

*P <0.05. was considered as significant. Data are expressed as Percent of relative frequency of gastrointestinal symptoms. P-value within group comparison of variables resulted from Chi-Square Tests.

The numbers 0, 1 and 2 indicate the severity of gastrointestinal symptoms. 0; the patient hadn’t gastrointestinal symptoms, 1; patient had occasional gastrointestinal symptoms. 2 ≤; the patient had permanently gastrointestinal problems

### Additional File

Additional file 1: Standard Protocol Items: Recommendations for Interventional Trials (CONSORT) 2010

Checklist: recommended items to address in a clinical trial protocol and related documents.

### Figures
Figure 1

Stages of clinical trial progress

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

Tables.pdf
CONSORT 2010 Checklist.pdf
