The effects of Bunium Persicum (Black Caraway) supplementation on glycemic indices, lipid profiles and serum levels of nesfatin-1 in overweight and obese patients with type 2 diabetes: a double-blind randomized placebo-controlled clinical trial

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Abstract

Background: Diabetes mellitus is the most common metabolic disorder worldwide. Our aim was to determine the effects of bunium persicum (BP) on serum glucose indices, lipid profile, and nesfatin-1 levels among overweight or obese T2DM patients.

Methods: The place of participant recruitment was the diabetic clinic of Bu-Ali hospital in Zahedan. Based on the eligibility criteria, 60 participants were randomly divided into two groups as BP (n=30) or placebo (n=30). The supplementation was one 1000 mg capsules 2 times/day BP with launch and dinner for 8 weeks. Bodyweight, Waist circumference, serum nesfatin-1, fasting blood sugar (FBS), insulin (FBI), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured. Quantitative insulin sensitivity checks index (QUICKI), homeostasis model assessment-insulin resistance (HOMA-IR) and Body Mass Index (BMI) were also calculated.

Results: In comparison with placebo, PB significantly increased QUICKI and decreased FBS, HOMA-IR, BMI and WC ($P<0.05$). At the end of the study after adjustment for confounders, the changes were similar ($P<0.05$) with an exception for QUICKI which had a trend ($P=0.054$) and WC ($P > 0.05$). The differences in the FBI, TG, TC, LDL, HDL and Nesfatin-1 were not significant ($P>0.05$).

Conclusion: PB supplement improved serum glucose indices and decreased BMI among overweight or obese T2DM patients; though, further trials are suggested to confirm results.

Background

Diabetes is a multifactorial autoimmune disorder, which is determined by a high blood glucose level and a leading cause of death worldwide. The prevalence of diabetes mellitus type 2 (T2DM) is increasing globally and its complications are a major health problem [1, 2]. In 2000, over 171 million were suffered from diabetes and it is estimated that the prevalence of the common and important disease will exceed 366 million patients with an increase in mortality rate 30 years later [3]. Some metabolic perturbations such as B-cell dysfunction, impaired insulin secretion, and insulin resistance contribute to the pathogenesis of diabetes [4].

Management of diabetes to prevent its complexity and quality of life of diabetic patients becomes

achievable with applicable intervention and alterations in lifestyle, including changes in dietary pattern, regular physical activity, and anti-diabetic medications [5, 6].

Various types of traditional, complementary or alternative therapies have been increasingly used for various chronic diseases [7, 8], such as diabetes and hyperlipidemia in all around the world [9]. Herbal medicines are a major part of these therapies [10]. The World Health Organization has encouraged and recommended that herbal therapy for diabetes needs further assessment [11]. One of the medicinal plants, which have been getting enhancing attention lately, is Bunium persicum (BP). Bunium persicum, belonging to the Apiaceae family, is a widely distributed annual herbaceous plant [12]. Its seed is generally known in Iran as 'Zireh Siah' and has been used extensively in local foods and Iranian traditional medicine to cure several disorders [13].

Furthermore, this plant is called with different names throughout the world as ''Black zire'','' Black caraway", ''Carum carvi'', ''Persian Cumin'', "Zire kuhi", " Shah zira "," Kala Zeera", "Black Persian cumin", "Wild caraway" and "wild cumin"[14, 15].

This herb has been used since ancient times, particularly in the treatment of digestive disorders and is known worldwide as antibacterial, antiulcerogenic, and is traditionally used to treat flatulence, colic pain, bronchitis, diabetes, cardiovascular diseases, hypertension, and some gastrointestinal illnesses, including diarrhea and inflammatory bowel disease [16-18]. Furthermore, it may serve as a dietary source of natural antioxidants for health improvement and may be used as a natural antioxidative food additive to increase food quality and stability [19]. The known main constituents of BP have been demonstrated as gamma-terpinene, p-cymene, cuminal and cumin aldehyde [20, 21].

Nesfatin-1 is a newly identified 82 amino acid peptide derived from its greater precursor protein, NEFA/nucleobindin 2 (NUCB2) [22]. Nesfatin-1 dramatically decreases food intake when administered into the third cerebral ventricle of rats at picomole doses. The result of a recent report by Shimizu showed the potential physiological relevance of endogenous nesfatin-1 in the prevention of food intake was suggested by the stimulation of food intake induced by I.C.V injection of a nesfatin-1

antibody [22, 23]. fasting nesfatin-1 was significantly decreased in T2DM patients compared to healthy subjects and maybe one of the appetite-related hormones involved in diabetic Polyphagia [24]. Accordingly, this study is based on the hypothesis that bioactive compounds found in Bunium Persicum have antidiabetic and Appetite-Suppressing properties. The hypoglycemic, antihyperlipidemic and appetite reducing effects of caraway in humans and rats have been shown before [13, 25]. Because bunium persicum is used widely in Iranian traditional medicine and as a common spice in Iranian foods, and to the best of our knowledge, there had been no clinical study on the effect of Bunium persicum on metabolic status of overweight and obese patients with type 2 diabetes and also, previous related studies have reported some inconsistent results; the present study was conducted to evaluate the effect of Bunium Persicum powder on glycemic indices, lipid profiles and serum levels of nesfatin-1 in overweight and obese patients with type 2 diabetes.

Methods

-Study Design and subjects:

The ethics committee of Zahedan University of Medical Sciences approved this double-blind randomized placebo-controlled clinical trial under the code of IR.ZAUMS.REC.1397.332. The study was registered to Iranian Registry of Clinical Trials as IRCT20181207041876N1 on 18/01/2019. The participators were overweight or obese T2DM patients referring to the diabetic clinic of Bu-Ali Hospital of Zahedan. This trial lasted from 23 June 2019 until 22 October 2019.

Inclusion criteria were T2DM diagnosed, age 30–65 years, and $25 \leq BMI < 40$ kg/m^2. On the other hand, **Exclusion criteria** were Suffering from cognitive disease or other psychotic illnesses, depression, Acute systemic disease, cystic fibrosis disease, muscular dystrophy, protein malnutrition, a history of gastrointestinal surgery, neurological disorders, structural abnormalities of the gastrointestinal tract, disability, uncontrolled hypertension (>140/90 mmHg), Treatment with statins, antihypertensive, probiotics, multivitamin-mineral and antioxidant supplements during the three past months, pregnancy or lactation, expert athlete, intake of statins, caraway interacting drugs, losing weight during the past 3-months and not taking more than 10% of the intervention supplement.

This trial adhered to CONSORT guidelines and included a completed CONSORT checklist as an additional file.

-Randomization and Intervention:

According to the block randomization method, participants were divided into two equal groups by an assistant (BP [n=30] or placebo [n=30] groups). The stratified randomization method was utilized for matching age (30-45 and 46-65 yrs) and gender. The ratio of the two groups was 1:1. Three patients from the PB group and 2 patients from the placebo group declined to participate after randomization and before the beginning of the study (Figure 1).

The blinding of intervention allocation was done for both the participants and investigators as A and B packages. The PB and placebo capsules were made by the Herbarium center of the Kerman Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran. The shape, size, and color of the capsules were similar. The contents of capsules were 1 g of whole PB or Starch powder. Before supplementation, the capsules were weekly placed alongside each other to get a similar smell. The absorbed Bunium Persicum volatile oil in the placebo capsules was very low to change health parameters. According to a previous study, the dose of supplements was determined 2 grams/day as 2 capsules per meal with food [26]. However, the intake and absorption of the PB either with or without food should be investigated further. The supplements were distributed monthly basis and checking compliance status was also done monthly through face to face and weekly by telephone. The duration of the intervention period was 8 weeks.

The PB voucher number was *Bunium persicum (Boiss) B. Fedtch, Family: Apiaceae, KF1141-1*. The analysis of the whole PB was performed by the Herbarium center of the Kerman Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran. The contents of Bunium persicum essential oil according to the gas chromatography-mass spectrometry (GC-MS) were 46.1 % γ-Terpinene and 15.5 % cumin aldehyde.

-Assessments and Measurements:

***General Characteristics, Dietary Intakes, and Physical Activity*:**

The T2DM patients were identified, the eligibility criteria checked, the study details were clarified, and

informed consent was obtained by the main investigator (BSC student). The questionnaires including the general questionnaire, 24-hour food recall (at the beginning and end), and short-form IPAQ (SF-IPAQ) questionnaire (at the beginning and end) were administered through interviewing.

The dietary status was determined using gram per day of values from 24-hour food recall (valid in Iran [27]) by the *Nutritionist 4* software [27, 28].

The IPAQ questionnaire provides information on physical activity that people do as part of their everyday lives. The questions are about the time when the person has been active during the last 7 days. This questionnaire addresses the activities in the workplace or as part of the homework and the garden, place to place movement, exercises, and leisure activities. It also considers all the intense activities over the past 7 days. Intense activities require a great deal of physical power and more intense breathing. The IPAQ addresses only continuous activities for at least 10 minutes. Its short form has 7 classified questions determining the three activity levels (1-3 or low-to-high levels). This questionnaire had been validated in Iran [29, 30].

***Anthropometric measurements*:**

Weight (at the beginning and end), height (at the beginning) and waist circumference (at the beginning and end) were determined by using a digital scale and stadiometer (*Seca® Germany, Model: 755 1021994*). They were measured as such: Weight without shoes, with minimal clothing, and with 100-gram accuracy; height without shoes, standing, heels sticking to the wall, flat and forward head, and with 0.5-centimeter accuracy; and waist circumference with minimal clothing, at the middle of the last rib and the iliac crest. Body mass index (BMI) was calculated via dividing weight in kilograms by squared height in meters.

***Blood biomarkers measurement*:**

At the beginning and the end of the study, 10 ml of blood (at the beginning and end) was taken from the peripheral vein after 12-hour fasting during the night and centrifuged for 20 min (3000 *g*). Serum glucose was determined on the same day of blood withdrawal. The remaining serums were frozen and stored at -80°C up to the analysis.

Serum nesfatin was determined using the sandwich ELISA and kit as *Shanghai Crystal Day Biotech Co. Ltd®; Intra-assay CV<8 %, Inter-assay CV<10* % by an automatic device (*Elisys Uno Human®*). Similarly, the ELISA kit for FBI was *DiaMetra® Co of Italy, DCM076-8; Intra-assay CV≤5 %, Inter-assay CV≤10 %.* Serum glucose was measured according to the glucose oxidase method using the Hitachi analyzer device (*q17®*) and the specific kit as *Bionik®, Liquid Stable, Glucose oxidase GOD-POD, Mono-reagent; Intra-assay CV≤2.10 %, Inter-assay CV≤3.09 %*. Also, the serum levels of TC, TG, LDL-C, and HDL-C were measured using Hitachi analyzer device (*q17®*) and the specific kits as 1-*Bionik®, Liquid Stable, Enzymatic Colorimetric CHOD-POD*, 2-*Bionik®, Liquid Stable, GPO-POD, Mono-reagent*, 3-*Bionik®, Liquid Stable, Direct. Enzymatic Colorimetric*, and 4-*Bionik®, Liquid Stable, Direct. Enzymatic Colorimetric*, respectively. The intra- and inter-assay coefficients of variation for TC, TG, LDL-C, and HDL-C were ≤1.216% and ≤6.906%, ≤1.573% and ≤7.704%, ≤1.76% and ≤0.65 %, and ≤0.7% and ≤1.5%, respectively. HOMA-IR and QUICKI indices were calculated by the following formulas:

QUICKI *= 1 / (log fasting insulin [µIU/ml]) + log fasting glucose [mg/dl])*

HOMA-IR *= FBI [µIU/ml] × FBS [mg/dl] / 405*

Sample Size:

as regards, no article was found similar to our study, the sample size determined by considering the sample size of the similar studies in obese and overweight patients [31, 32] and using the extract of anti-obesity factors [25], The sample size determined from 8 to 44 in each group. The sample size for this study according to study limitations (control of various factors such as powder consumption, diet control, physical activity, nutritional counseling, cost) in each group 27 patients are considered but accounting to 10% missing during the study, the number of patients is 30 in each group.

-Data Analysis and Accessibility:

Data management including entry, security, coding, and storage was performed at this stage. The missing data of the follow-up stage and baseline of one patient were estimated by modified-intention

to treat (m-ITT) analysis and regression imputation method. The Kolmogorov-Smirnov, Chi-square, Fisher Exact, and t or Mann-Whitney tests assessed normality of continuous variables as well as categorical and continuous baseline characteristics, respectively. Two-way repeated measures analysis of variance (TWRM-ANOVA) was used to determine time effects and time by treatment interaction effects on all dependent variables. Moreover, TWRM-ANOVA was adjusted for dietary intake of vitamin B12. Also, 95% confidence interval (CI) and a P-value<0.05 were considered for reporting the measurements. Data analysis was conducted using $SPSS_{16}$ (statistical package for the social sciences) and $STATA_{11SE}$ (general-purpose statistical software package by Stata Corp) software. The main investigator had access to the final dataset and the results were presented by the publication.

Results

-Participants' characteristics

According to Figure 1, overall, 680 people were screened based on medical history. Specifically, 82 subjects had the eligibility criteria, of whom 12 declined and 4 could not participate. Also, 66 subjects were randomized, with 3 subjects in the BP group and 3 subjects in the placebo group refusing to participate and as such did not receive the intervention. Thus, the first visit was completed for 60 subjects (bunuim persicum n = 30; placebo n = 30). In addition, 6 subjects could not continue the follow-up stage (for personal reasons and travel; bunuim persicum n = 3; placebo n = 3). Further, the baseline serum sample of two subjects in the placebo group was not available. Eventually, data analysis was performed for 52 subjects according to the modified-ITT analysis.

The general characteristics and physical activity level of the patients are presented in Table 1. Most of the participants had similar education, high economic and low physical activity level. Both groups used more than 95% of the prescribed supplements.

- Changes in dietary intake and blood biomarkers

The dietary intake of carbohydrates, protein percent and iron in the baseline were higher in the BP group, while the other baseline features were similar between the two groups (Tables 3).

The dietary intake of vitamin B12 during the study was higher in the BP group (P<0.05, Table 3),

while the other dietary intakes were almost similar between the two groups. This significant intake was considered as a confounder in the final analysis model. Within the BP group, the mean difference of TC, TG, HDL and LDL were not significant (P>0.05). On the other hand, FBS, FBI, BMI, WC and HOMA-IR reduced, while QUICKI and nesfatin-1 increased significantly (P<0.05). Within the placebo group, the mean differences of FBS, FBI, HOMA-IR, QUICKI, TC, TG, LDL-C, HDL-C and nesfatin-1 were not significant (P>0.05) (Table 4).

According to the time by treatment interaction effect in the final analysis model, FBS, HOMA-IR, BMI, and WC declined while QUICKI grew significantly among the BP group in comparison with the placebo group (P<0.05) (Table 4). In other words, BP in comparison with placebo significantly elevated QUICKI, reduced FBS and HOMA-IR (P<0.05). After adjustment for confounders, the significant changes were similar (P<0.05) with an exception for WC (P=0.2) (Table 4).

-Safety

Any side effects related to the treatment were reported, and only vomiting was observed for one patient in the BP group in one of his follow-ups.

Discussion

This trial is the first to assess the beneficial effects of Bunium persicum on blood glucose indices, lipid profile and Serum levels of nesfatin-1 in overweight or obese patients with type 2 diabetes mellitus (T2DM). The different clinical usages and the lack of awareness concerning the advantages and disadvantages of Bunium Persicum in patients with T2DM made this study very pertinent. According to both unadjusted and adjusted analysis models, consumption of 2,000 mg of BP for 8 weeks caused a significant reduction in FBG, HOMA-IR, BMI and augmented QUICKI in the intervention group compared with placebo. Furthermore, the decrease in WC was significant in the unadjusted model but not significant in the adjusted model. Limited human studies have investigated the effect of Bunium persicum on glycemic indices and lipid profiles and according to our knowledge, no study that examined the effects of Bunium persicum on serum Nesfatin-1 level in these patients.

However, some studies have been conducted on the effect of caraway (Bunuim Persicum and Carum Carvi) on diabetic rats. In one of these studies, no significant differences were seen in terms of dietary

intake between the groups before and after the intervention except vitamin b12. In another study by Kazemipoor et al., no significant differences were found in dietary intake at baseline, but at the end of the intervention, the results showed a significant reduction in carbohydrate intake in the caraway group [25].

The means of FBS levels decreased from 175.4±69.9 mg/dl to 142.6±53.8 mg/dL in BP group ($p < 0.05$). The decrease in FBS was significantly observed at the intervention group compared with the placebo ($p < 0.05$). Likewise, caraway consumption leads to a reduction of fasting blood insulin levels in the intervention group from 9.4±6.8 to 5.6±2.5, ($p <0.05$) and HOMA-IR from 4.2±3.7 to 2.3±1.3 ($p <0.05$). Even though, the mean differences of the FBI were not significant in the intervention group as compared to the control group at the end of the study ($p>0.05$). The blood glucose lowering effect of Bunium persicum is maybe for the prohibition of hepatic glucose production or stimulation of glucose usage by muscle and adipose tissue [33] or inhibition of glucose reabsorption in the kidney [34]. Another hypothesis for the possible mechanism of hypoglycemic activity of this plant may be through its main bioactive compounds. The main components of bunuim persicum (KF1141) oils were γ-terpinene, cumin aldehyde, ρ-cymene and limonene [35]. The hypoglycemic effect of limonene has been previously reported in diabetic rats by decreasing the activities of gluconeogenic enzymes, increasing the glycolytic enzymes and stimulate insulin secretion in pancreatic β-cells [36, 37]. Soundharrajan et al (2018) indicated that Limonene like insulin, could stimulate glucose absorption in differentiated cells through 2-Deoxy-D-glucose uptake by activation of complex Protein kinase B signaling pathways [38]. D-limonene can significantly decrease the activities of glucose 6-phosphatase and fructose 1, 6-bisphosphatase which can lead to decreased gluconeogenesis and thereby reducing the endogenous production of glucose(39). The presence of terpenoids is more significant in bunium persicum since they are reported to possess hypoglycemic activity in diabetic and normal mammals. The results revealed that the methanolic extract of Bunium persicum showed 36% inhibition in α-amylase at a concentration of 100 mg/mL. High differences were found in the activity of ethyl acetate extracts of Bunium persicum which showed 40% of inhibition at 250 mg/mL, respectively. Although,

the mechanism of action is not clear(15, 40). Also, cumin aldehyde in the bunium persicum oilseeds has a significant inhibitory activity against the α-glucosidase enzyme that catalyzes the final step in the digestive process of carbohydrates. Its inhibitory effect of cuminaldehyde can postpone glucose uptake and reduce hyperglycemia [41]. The hypoglycemic effect of caraway seed has been previously shown by investigators. Haidari et al reported that oral consumption of caraway caused a significant decrease in blood glucose levels in streptozotocin-induced diabetic rats [13]. Kazemipoor et al. (2015) studied the consumption of Caraway extract in overweight and obese women patients. After 12 weeks, no significant effect was found for FBS levels [42]. In another research by Seyed Sadjadi et al. (2014), Short-Term Extract Administration of Caraway significantly decreased FBS levels in STZ-induced diabetic rats compared with control diabetic rats [43]. The anti-hyperglycaemic effect of caraway has been shown by M. Eddouks et al in diabetic rats previously. Oral administration of the aqueous caraway extracts (20 mg/kg) significantly decreased blood glucose levels in STZ diabetic rats but no changes were observed in fasting plasma insulin concentrations after treatment in either normal or STZ diabetic rats. these results indicated that the underlying mechanism of this pharmacological activity seems to be independent of insulin secretion [44]. Nevertheless, controversial results of some studies showed that consumption of Ethanolic Extract of caraway and Bunium Persicum Seeds significantly increased fasting blood insulin levels in streptozotocin-induced diabetic rats [45, 46].

In this study, the effect of caraway powder consumption on TG, cholesterol, HDL and LDL levels was not significant. Similarly, Kazemipoor et al. (2015), reported that consumption of caraway extract in overweight and obese women was not effective on lipid profiles [42]. Ghorbani et al. (2017) investigated the effect of high intensity interval training along with consumption of caraway seeds (Carum carvi L.) on liver enzymes, lipid profile, and blood glucose in obese and overweight women and found significant differences in triglyceride($p=0.043$) between groups and Paired t-test results showed that there is a significant difference between pre and post levels of triglyceride in overweight and obese groups, but no significant difference was observed in the other variables between the

groups. However, HDL was significantly increased in the overweight group compared to the control and obese groups [31]. Our results are not consistent with the various studies on animals that have shown that caraway (bunium persicum and C. carvi) has significant hypolipidemic effects [43, 47-49]. In the study of Lemhadri et al., It was found that the extract of caraway produced a significant decrease in triglycerides levels at normal rats and a decrease in cholesterol levels in STZ diabetic rats. On the other hand,15 days after repeated oral administration of caraway extract exhibited a significant triglyceride and cholesterol-lowering activities In both groups of normal and STZ diabetic rats [48]. In another animal study, there was a significant inverse relationship between oral administration of caraway and total cholesterol ($p < 0.05$) and low-density lipoprotein levels ($p = 0.001$) in the treated animals compared with the diabetic control rats. but no significant changes were reported in the levels of high-density lipoprotein and triglycerides [13].

With regard to the findings of the present study, caraway significantly reduced body weight, Body Mass Index and Waist Circumference compared with the placebo group. Results in our study are almost consistent with the results of the Kazemipoor et al. (2015) study, During the 3-month intervention, the waist circumference (WC), the mean body weight and BMI had significantly decreased in the caraway extract group compared with the placebo group [42]. In another human study in obese women, Mohammadkhani et al. (2019) found that 8 weeks of aerobic exercise and taking a caraway supplement can improve weight and WHR in the supplement-exercise group compared with placebo-exercise and control groups [50]. Recently, Khaksari et al reported no significant changes in body weight after 6 weeks of Bunium persicum aqueous extract administration in hypercholesterolemic male mice [47].

Weight, BMI and WC lowering effect of BP in our study may be related to Nesfatin-1 hormone levels that improved within the BP group in this study ($p < 0.05$). Nesfatin-1 may contribut to the energy homeostasis, decrease appetite and reduce body fat mass [51-53]. Thus, there is a possibility that nesfatin-1 plays a role in the decrease in body weight in the BP group of our study [51]. In line with

our findings, A.Stengel et al reported that injection of nesfatin-1 can reduce body weight in rats [54]. Also intracerebroventricular (ICV) and peripheral nesfatin-1 injection in rats can inhibit food intake [55, 56].

The novelty would make this study very relevant. As the side-effects of the PB (up to 2 gram/day) had not been reported previously, it may be practically feasible for patients to continue taking it in the long run. Nevertheless, the effects and durability of this intervention in the long run should be investigated. The use of the PB in some diseases especially T2DM needs to be further studied. In addition, the emergence of obesity and, consequently, T2DM should also be considered.

The important strengths of this study were: the earliest assessment of PB effects in overweight or obese T2DM patients especially by assessing nesfatin-1 levels, the double-blinded stratified blocked randomization design and assessing dietary intakes and physical activity status and adjusting for them. However, some limitations were the self-reporting of diet and physical activity, measuring hemoglobin A1c (HbA1c), body composition, and bioavailability and serum levels PB or its components, and determining the durability of the intervention in the long run, and 24-hrs food recall which is not appropriate for determining the usual food intake.

Conclusion

PB supplementation in overweight or obese T2DM patients showed a significant beneficial effect on serum glucose indices and BMI. Further trials are required to use PB in clinical practice.

Abbreviations

T2DM: type 2 diabetes mellitus, BP: Bunium persicum, BMI: body mass index, IPAQ: international physical activity questionnaire, TMRC: Traditional Medicine Research Center, HPLC: high-performance liquid chromatography, I.C.V injection: Intracerebroventricular injection, TG: triglyceride, ELISA: enzyme-linked immunosorbent assay, TWRM-ANOVA: two way repeated measures analysis of covariance, ITT: intention to treat

Declarations

-Ethics approval and consent to participate: This trial was approved by the Ethics Committee of Zahedan University of Medical Sciences (Ethical Code: IR.ZAUMS.REC.1397.332). A written informed

consent form (in Persian) obtained from all patients. Participation was free, and a patient could withdraw at whatever point the person feels he/she was unable to continue. The lifestyle advice was presented free to the patients and there was no bar to receiving the other health care services of the center. Side-effects of Bunium Persicum (Black Caraway) supplement (up to 2 gram/day) have not been reported previously. The personal information of patients was kept secret before, during, and after the study.

-Consent to publish: Not applicable.

-Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on a reasonable request.

-Competing Interests: There is no potential conflict of interests with respect to research, authorship, and publication.

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-Authors Contributions: SJM, MS, MDM, and ZM conceived and developed the idea for the paper and revised the manuscript. ASG wrote numerous drafts. ADP contributed to statistical interpretations. All authors read and approved the final manuscript.

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Tables

Table 1. General characteristics and physical activity of overweight or obese patients with type 2 diabetes mellitus (T2DM)

General variables and physical activity		Caraway (n=27) n(%) or Mean(SD)	Placebo (n=25) n(%) or Mean(SD)	P
Age (yrs)	30-45	12(46.2)	9(45.5)	
	46-60	14(53.8)	12(54.5)	
Gender	male	8(30.8)	9(42.9)	
Education level	up to associate degree	11(42.3)	10(47.6)	
	Bachelor and higher	15(57.7)	11(52.4)	
Economic level	Low/moderate (≤6 living items)	7(26.9)	6(28.6)	
	High (≥7 living items)	19(73.1)	15(71.4)	
Physical activity level (Baseline)		1.4(0.6)	1.3(0.5)	
Physical activity level (End)		1.3(0.6)	1.4(0.5)	

*Mann-Whitney; **Chi-square; **$**t-test

Table 2. Comparison of baseline mean for BMI and serum nesfatin-1, glucose indices, and lipid profile in overweight or obese patients with type 2 diabetes mellitus (T2DM)

Baseline Dependent Variables		Caraway (n=27) n(%) or Mean(SD)	Placebo (n=25) n(%) or Mean(SD)	P-value
BMI (kg/m2)	25-29.99	19(73.1)	15(71.4)	0.9$
	30-34.99	7(26.9)	6(28.6)	
FBS (mg/dl)		175.3(17.6)	174.0(17.9)	0.9*
FBI (µIU/ml)		9.4(1.6)	11.6(1.9)	0.3*
HOMA-IR (score)		4.2(3.0)	4.9(3.7)	0.2**
QUICKI (score)		0.33(0.004)	0.31(0.002)	0.1*
Nesfatin-1 (ng/ml)		4.4(1.6)	5.5(2.1)	0.1*
TC (mg/dl)		159.0(36.0)	152.6(48)	0.6*
TG (mg/dl)		163.3(117.1)	164.2(82.4)	0.9*
LDL-C (mg/dl)		81.3(27.6)	83.3(29.6)	0.8*
HDL-C (mg/dl)		45.0(12.0)	43.0(7.6)	0.5*

*t-test; **$**Chi-square; **Mann-Whitney; **BMI**: body mass index, **HOMA-IR**: homeostasis model assessment-insulin resistance, **QUICKI**: quantitative insulin sensitivity check index, **FBS**: fasting blood sugar, **FBI**: fasting blood insulin, **TC**: total cholesterol, **TG**: triglyceride, **HDL-C**: high-density lipoprotein cholesterol, **LDL-C**: low-density lipoprotein-cholesterol

Table 3. Mean of dietary intakes during the study on overweight or obese patients with type 2 diabetes mellitus (T2DM)

Dietary intakes during the study	Bunium persicum (n=27) Mean(95% CI)	Placebo (n=25) Mean(95% CI)	P-v
Energy (kcal)	1982.6(1951, 2014.2)	1799.3(1793.4, 1805.3)	(
Protein (g)	72.1(71.6, 72.6)	77.8(78.9, 76.8)	(
Protein (%)	14.4(14.5, 14.3)	17.3(17.5, 17.2)	(
Carbohydrate (g)	274.6(268.6, 280.7)	227.0(230.7, 223.3)	(
Carbohydrate (%)	54.8(54.5, 55.1)	49.6(50.4, 48.8)	(
Fat (g)	69.1(69.0, 69.3)	66.5(65.3, 67.8)	(
Fat (%)	30.8(31, 30.6)	33.0(32.0, 34.1)	(
Cholesterol (mg)	133.4(123.5, 143.3)	130.9(132.3, 129.5)	(
Saturated fat (g)	20.3(19.6, 21.1)	16.1(15.9, 16.3)	(
Monounsaturated fatty acid (g)	25.3(26.9, 23.7)	24.4(25.0, 23.9)	(
Polyunsaturated fatty acid (g)	17.0(16.4, 17.6)	17.8(17.7, 18.0)	(
Vitamin A [RAE] (µg)	806.2(884.3, 728.2)	676(752.7, 599.3)	(
Vitamin C (mg)	149.5 ,123.3)4.136)	132.6 ,118.2)4.125)	(
Potassium	2708.6(2625.0, 2792.3)	2588.1(2540.4, 2635.8)	(
Calcium (mg)	823.4(837.1, 809.8)	911.4(934.2, 889.4)	(
Iron (mg)	19.4(19.1, 19.7)	15.2(15.2, 15.3)	(
Vitamin D (µg)	0.6(0.5, 0.7)	0.5(0.4, 0.6)	(
Vitamin E (mg)	17.5(19.8, 15.3)	16.0(16.6, 15.4)	(
Vitamin B1 (mg)	1.9(2.1, 1.7)	1.8(1.9, 1.8)	(
Vitamin B2 (mg)	1.8(1.9, 1.7)	1.8(1.9, 1.7)	(
Vitamin B3 (mg)	23.3(23.9, 21.7)	25.7(25.6, 25.9)	(
Vitamin B6 (mg)	1.7(2.5, 2.4)	2.8(3.3, 2.4)	(
Folate (DFE) (µg)	143.7(138.3, 149.2)	144.7(147.5, 141.9)	(
Vitamin B12 (µg)	2.5(2.4, 2.7)	2.1(2.3, 1.9)	
Vitamin K (µg)	182.0(129.3, 234.7)	154.9(106.8, 203.0)	(
Zinc (mg)	9.5(9.5, 9.6)	8.6(8.4, 8.8)	(
Selenium (µg)	33.1(32.2, 34.1)	39.2(39.0, 39.4)	(
Total fiber (g)	4.7(4.4, 5)	4.1(3.8, 4.5)	(

*Two way repeated measures-ANOVA (TWRM-ANOVA)

Table 4. The changes of BMI,WC,serum nesfatin-1, glucose indices, and lipid profile in overweight or obese patients with type 2 diabetes mellitus (T2DM)

Variables	Supplement	Baseline Mean	End Mean	P-value $	Mean Changes (95 % CI)	P-value#		
						Time	Treatment	Interaction
BMI	Caraway (n=27)	28.8(3.8)	28.3(4.0)	0.003	-0.5 (0.2, 0.7)	0.4	0.5	0.02
	Placebo (n=25)	29.2(3.3)	29.4(3.3)	0.4	0.2 (-.8, 0.3)	0.8	0.6	0.04
WC (cm)	Caraway (n=27)	100.3(7.0)	99.0(7.4)	0.002	-1.3 (0.5, 2.0)	0.013	0.8	0.04
	Placebo (n=25)	100.3(8.4)	100.2(8.3)	0.7	-0.1 (-0.7, 0.9)	0.1	0.6	0.2
FBS (mg/dl)	Caraway (n=27)	175.4(69.9)	142.6(53.8)	0.002	-32.8 (11.9, 51.2)	0.036	0.3	0.005
	Placebo (n=25)	174.0(52.3)	178.9(73.5)	0.6	4.9 (-22.7, 12.9)	0.2	0.8	0.02
FBI (µIU/ml)	Caraway (n=27)	9.4(6.8)	5.6(2.5)	0.03	-3.8 (0.3, 7.3)	0.245	0.006	0.15
	Placebo (n=25)	11.6(7.5)	12.0(9.0)	0.9	0.4 (-5.1, 5.3)	0.140	0.01	0.05
HOMA-IR	Caraway (n=27)	4.2(3.7)	2.3(1.3)	0.041	-1.9 (0.9, 3.8)	0.181	0.009	0.047
	Placebo (n=25)	5.0(3.1)	5.2(4.7)	0.87	0.2 (-2.5, 2.1)	0.173	0.034	0.018
QUICKI	Caraway (n=27)	0.45(0.03)	0.47(0.03)	0.006	0.02 (-.03, 0.0)	0.014	0.128	0.035
	Placebo (n=25)	0.444(0.02)	0.446(0.03)	0.761	0.01 (-.04, 0.1)	0.050	0.4	0.054
TC (mg/dl)	Caraway (n=27)	159.0(36.8)	159.5(37.1)	0.942	0.5 (-13.3, 12.4)	0.4	0.8	0.5
	Placebo (n=25)	152.6(49.0)	159.9(45.2)	0.395	7.3 (-24.9, 10.3)	0.8	0.6	0.9
TG (mg/dl)	Caraway (n=27)	163.3(117.2)	175.3(121.4)	0.389	-12.0 (-39.9, 16.1)	0.1	0.9	0.8
	Placebo (n=25)	164.2(82.4)	179.3(100.4)	0.189	15.1 (-38.1, 8.0)	0.3	0.9	0.8
LDL-C (mg/dl)	Caraway (n=27)	81.3(27.6)	82.6(27.7)	0.842	1.3 (-13.7, 11.3)	0.6	0.9	0.4
	Placebo (n=25)	83.3(29.6)	77.8(39.3)	0.422	-5.5 (-8.5, 19.5)	0.9	0.4	0.1
HDL-C (mg/dl)	Caraway (n=27)	45.0(12.0)	44.2(11.2)	0.520	-0.8 (-1.6, 3.2)	0.9	0.7	0.3
	Placebo	43.0(7.6)	44.0(9.2)	0.51	1.0	0.7	0.8	0.3

	(n=25)			8	(-4.3, 2.3)			
Nesfatin-1 (ng/ml)	Caraway (n=27)	4.4(1.6)	6.1(3.4)	0.021	1.7 (-3.2, 0.3)	0.01	0.3	0.59
	Placebo (n=25)	5.5(2.1)	6.6(3.9)	0.168	1.1 (-2.9, 0.5)	0.2	0.7	0.8

$Paired t-test; #Two way repeated measures-ANOVA (TWRM-ANOVA), top row $P\text{-}_{value}$: unadjusted; bottom row $P\text{-}_{value}$: adjusted for vitamins B12 dietary intake

HOMA-IR: homeostasis model assessment-insulin resistance, **QUICKI**: quantitative insulin sensitivity check index, **FBS**: fasting blood sugar, **FBI**: fasting blood insulin, **TC**: total cholesterol, **TG**: triglyceride, **HDL-C**: high-density lipoprotein cholesterol, **LDL-C**: low-density lipoprotein-cholesterol

Figures

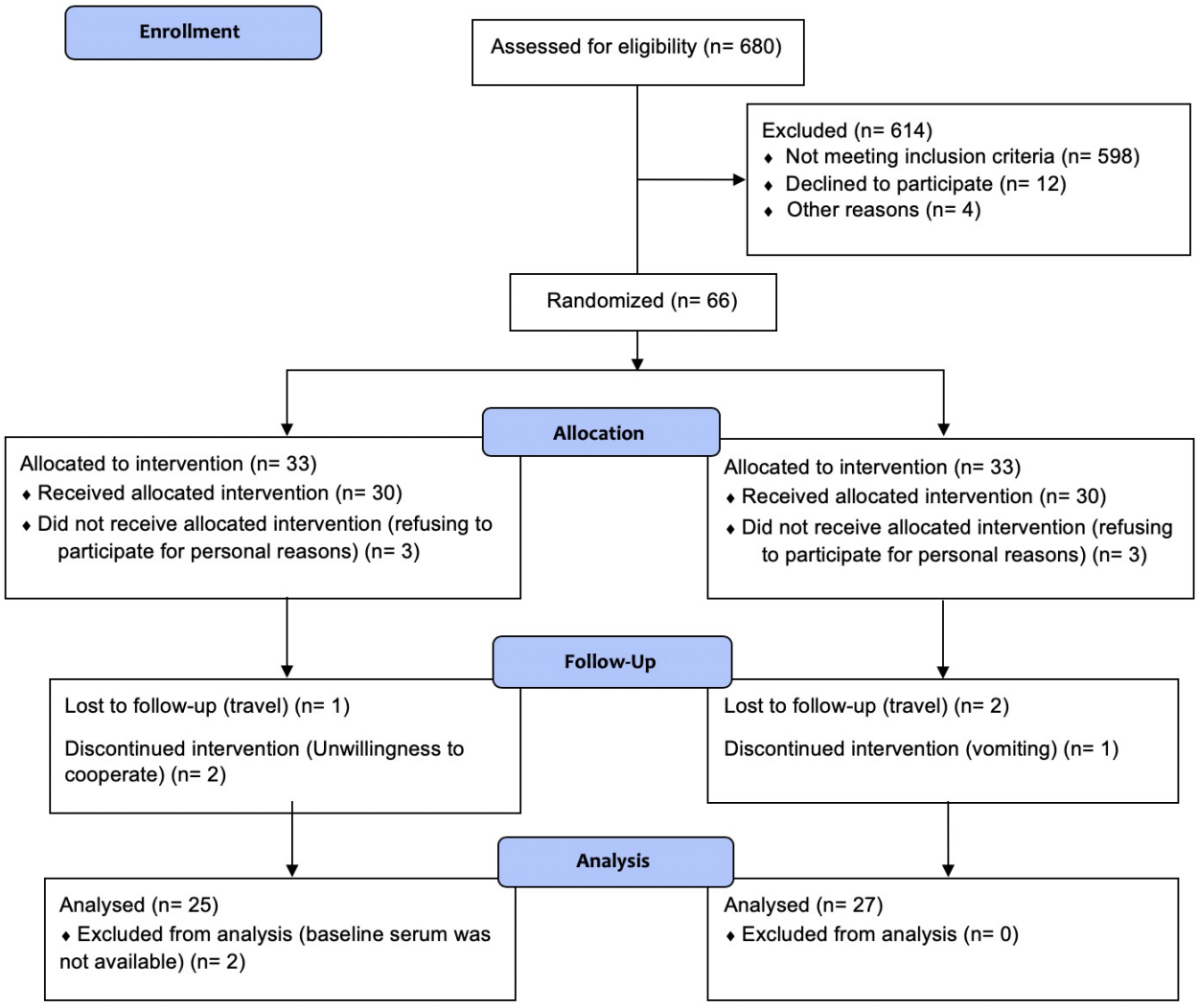

Figure 1

Flow chart of the study participants